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BIOLOGICAL SCIENCES CURRICULUM STUDY

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BIOLOGICAL SCIENCES CURRICULUM STUDY

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TEACHER'S MANUAL
for
STUDENT LABORATORY GUIDE

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ABOUT THE INDIAN EDITION

The Biological Sciences Curriculum Study comprises a group of American biologists who are interested in improving the teaching of biology in the high schools in the United States. In order to contribute to such an improvement the BSCS has prepared, with financial support from the U.S. National Science Foundation, a variety of printed materials of which *Biological Sciences: An Inquiry Into Life* is one example.

This version (consisting of a textbook, laboratory manual and a teacher's guide) was designed for American students. The high school teachers and college professors, who were the writers, had the domestic educational pattern in mind and placed primary emphasis on the North American fauna and flora.

It follows that we do not feel that the BSCS materials are appropriate for use in the classrooms of other nations without specific modification for the local biota, customs and educational systems. We do hope that Indian colleagues will prepare such an adaptation and we would feel privileged to work with them towards such a goal.

For the interim, the National Council of Educational Research and Training have suggested to us that it would be useful to have our books reprinted in India in a limited edition so that they could be generally available to Indian scholars and teachers. Thus, interested persons could review them while weighing the advantages and disadvantages of an adaptation. We were delighted with this suggestion by the NCERT and have been pleased to do what was necessary to make this possible.

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The exercises with black numbers are basic exercises; those with gray numbers are supplementary.

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INTRODUCTION

The laboratory is the scientist's workshop. Although reading and discussion are essential in science, it is in the laboratory that the truth of ideas is tested.

When modern science was getting underway, much scientific work was done in the outdoors, where nature could be observed firsthand; indeed, much biological work must still be done in the field. However as time passed, it became apparent that more precise measurements and more accurate observations could be made in a laboratory, where the scientific worker could control conditions so that comparisons could be made between situations differing only in one significant respect. That was the beginning of *experiment* (the experiment of the single variable), which made possible such certainty of conclusions that it became the preferred method of science. In the broad sense, wherever this method is employed, indoors or outdoors, there will be found the scientist's laboratory. There the work of science is done, its spirit abides, and its methods are transmitted from one generation to the next. Science is not really learned from books; it is learned by asking nature the right questions. The laboratory is thus an essential part of the biology course and the *Student Laboratory Guide* is designed to give the student a great variety of biological experiences. He will be able to test the accuracy of statements in his textbook. He will be able to prove to himself whether or not certain statements regarding living organisms are true. He will be able to participate in discovery and will get the feel of what it is like to be a scientist.

LABORATORY ORGANIZATION

The *Student Laboratory Guide* has a dual purpose: first, to allow the student to see what should be seen; and second, to let the student learn how to investigate a variety of biological problems. The guide contains ninety exercises. Maximum flexibility in selection is desirable; the supplementary exercises are not to be regarded as secondary or less preferable, but simply as additional exercises, or as substitutes for certain basic exercises. The number of exercises accomplished and those chosen will depend upon time, laboratory, equipment, and supplies available to the teacher. Every effort should be extended toward doing the basic exercises if at all possible for they form an integrated, nonrepetitive series of investigations of major concepts and principles.

Each of the laboratory exercises is organized by number and title. The first number always refers to the chapter in the textbook to which the exercise is related. It is not necessary, however, to read the textbook before undertaking related laboratory work. Many times it is advantageous and necessary to do the laboratory work before reading the textbook chapters.

The student will learn from his own laboratory experiences and will have a better opportunity to interpret and appreciate the significance of the textbook material.

In the laboratory guide most exercises begin with a short introduction, a stated purpose, and a list of materials to be used. Procedure to be followed in the performance of the exercise then follows. Here, and occasionally elsewhere, the student will find questions to be answered carefully and thoughtfully and to be written into a permanent notebook. The questions in the laboratory guide are indicated by heavy numbers enclosed in parenthesis – **(4)** – at the end of a question. You may wish to have students answer all the questions or only certain questions for each exercise. Or you may wish to assign questions selectively to individual students. The questions asked in each exercise vary from extremely easy to quite difficult. It is not necessary to assume that every student should be able to answer every question in every exercise, as the range of questions will be, in many cases, beyond the ability of some of the students in a class.

Reports

Extra time will be required of the teacher for guidance of student reports. The nature of the program calls for the students to spend considerable effort in making reports of their exercises. They will be making observations, analyzing data, drawing conclusions, and answering questions related to their observations. Although they will be doing less of the traditional drawing, labeling, and answering of *general* questions, the students will probably spend more than the usual amount of time upon *scientific communication*. If this time is to be well spent, the teacher will need to give considerable attention to its supervision. Each teacher should read carefully the instructions given in the introductions to the exercises in order to understand clearly what is recommended there. This does not mean that a teacher should feel constrained to follow an inflexible procedure, and avoid thinking of better ways of handling laboratory problems. It does mean that, in the eyes of those who have prepared these laboratory materials, the exercises will largely lose their effectiveness if sufficient effort is not devoted to guidance and supervision of reports. It is not intended that all the laboratory reports be corrected by the teacher. However, the teacher must try to evaluate the degree to which one of the foremost objectives of the course – perhaps the foremost objective – namely, the increased understanding of the nature of scientific investigation, is being attained. Such an evaluation will depend largely on examination of what the students write about their experiments, of how accurately and faithfully they record their data, of how well they are able to handle the data quantitatively and graphically, and of how well they draw valid scientific conclusions. For these reasons it is suggested that the students be given specific "student rules" about keeping a data book and, in addition, about keeping a looseleaf notebook in which brief, logical, and complete reports of each exercise are to be assembled.

Discussion

Time should be allowed regularly for full discussion of each exercise and the questions it elicits. Since it is not intended that all the laboratory reports be corrected by the teacher, and since many laboratory topics lend themselves to valuable class discussion, the student will have an opportunity to correct his own work in the course of a class discussion. Discussion may be handled effectively in the form of a seminar, especially when one student has done a different experiment from other students, or a different part of an experiment. *After* a student's report has been carefully prepared, he can be given an opportunity to tell the class what he did, what he observed, and what conclusions he drew from his data. The class can then discuss, question, and criticize. This type of training is invaluable.

Drawings

The laboratory guide is not designed as a workbook. The illustrations and charts are intended only as models for students and are to be copied by the student in his notebook. The authors of the laboratory guide feel that each individual teacher should decide upon the number and type of drawings and laboratory reports to be required. Drawings should be neat, legible, and accurate and should serve the purpose of elucidating the concept involved. They should not be assigned as busywork or artistic enterprises.

Student Assistants

Whenever practical, students should be given an opportunity to take part in the preparation of materials, equipment, and apparatus for the laboratory. By involving students in the planning, the teacher can provide them with better understandings of basic concepts of biology, as well as useful understandings of the preparations and steps essential to effective laboratory procedure.

The teacher can capitalize on the interest and willingness to work of small groups of students present in almost every class. These students often deem it a privilege and even an honor to be permitted to help with laboratory preparation. The routine work they do will not only make a real contribution, but will help them to realize that a certain amount of "dishwashing" is an inescapable part of operating a laboratory.

These student helpers should be selected by the teacher on an entirely informal basis, and never to the exclusion of other students who may later become interested in helping. These students should under no circumstances be considered monitors, and should not be asked to correct papers, score tests, read laboratory reports, or do anything else that might place them in a position of advantage over their classmates.

Students who studied biology as freshmen or sophomores, and who are now seniors (or in some cases, juniors) are often willing to become laboratory assistants during their free time. They can be assigned to laboratories on a somewhat more formal basis since they are not members of the classes they are to assist. Criteria for selection of these assistants might include (1) the student's interest in biology, (2) scholarship in biology and related subjects, (3) general scholarship, (4) free time, (5) willingness to work hard, (6) attitudes toward biology, (7) willingness to help other students, (8) ability to get along with others, and (9) general reliability.

Even with the help of a group of interested class members and of a senior assistant, there are still many problems associated with keeping a laboratory in top working condition. Each student should learn that care of the room, equipment, and materials is important.

None of the foregoing suggestions can replace the value of a well-organized stockroom or preparation room. Here each item has an assigned place for storage where it is always kept after being used. If each item has its own place it is not likely to be put away dirty or in unsatisfactory condition.

Looking ahead and anticipating needs are hallmarks of effective teaching, and doubly so of biology teaching with its necessary advance preparation for laboratory work involving the use of living materials. The ability and the willingness to look ahead and to act ahead cannot be replaced by any device or assistance. This is the teacher's own responsibility.

Another responsibility, strictly that of the teacher, is laboratory safety. No matter how much help he may have, the teacher is still solely responsible for the conduct and condition of his laboratory. It is therefore especially important that he select his student helpers with the greatest of care.

Student Rules for Laboratory Work

The following student rules should be observed properly. You may wish to make copies of them for distribution in the laboratory. They will guide students toward good habits of laboratory procedure and will enable them to get the most from their laboratory time. The time spent in the laboratory should be as productive of knowledge and scientific understanding as the same amount of time spent in reading the textbook.

Orderliness

1. Arrange the materials you need for a particular experiment in an orderly fashion. Order avoids confusion and waste of time.
2. Label all materials accurately.
3. After using laboratory material and equipment, return them to their proper places.
4. At the end of the laboratory period, leave your working space clean and in order.

Cleanliness

1. Wash used glassware and instruments with cleaning powder and rinse them in clear water. Soak stubborn stains (such as those sometimes found in pipettes and on slides) in water slightly acidified with dilute hydrochloric acid.
2. Where sterile methods are necessary, follow the exercise directions closely.

3. Clean the sink after use. Avoid disposing of any refuse in the sink. Wrap refuse in paper towels and place it in the designated container.
4. Wipe off work tables and sink tables with a sponge or damp paper towels.

Care of Living Animals

1. Provide food and water *daily* during weekdays. Arrangements will be made for care of the animals on weekends. Some animals will die if left without water for a twenty-four-hour period.
2. Clean cages *daily*.
3. Make certain that animals are securely housed, so that escape is impossible.
4. The use of living animals in the laboratory is to be considered a privilege. You are expected to treat these animals humanely. Cruelty is no part of the make-up of a true biologist.

Care of Plants

1. Water plants daily during weekdays. Arrangements will be made for their care on weekends. The soil should be kept moist but not wet. Pull up weeds as they appear.
2. Additional directions for plant care, when needed, will appear in the laboratory guide.

Care of Instruments and Apparatus

Much of the laboratory apparatus is expensive. Exercise great care in its use. "Playing around" has no place in a laboratory. One careless slip may mean injury to a fellow student or the loss of valuable material.

Performance of Work

1. Read in advance the exercise to be done, and arrive at the laboratory prepared for work. You will need the entire laboratory period for performing the experiment and for making observations.
2. Observe all instructions carefully. "One minute" means one minute by the second hand on a watch or clock. Only by accurate performance can accurate results be obtained. Work not done accurately is only wasted effort.

Record Keeping

Accurate record keeping is one of the most important phases of your work. It is imperative that records be made *at the time of observation* in a hard cover, permanent data book. Include the date and time of each observation. Drawings are also important parts of records. Often an accurate picture is worth many words. Develop the skill of making clear drawings—it takes a lot of practice.

Reports

A sample outline is suggested here for writing an account of laboratory work in your notebook.

1. Title
2. Purpose

3. Materials and equipment
4. Procedure
5. Data—may be in the form of tables or graphs
6. Discussion—analysis and interpretation of the data
7. Conclusions—a brief summary

If you are allowed to remove the pages from your laboratory guide, and interleaf them with your reports in your notebook, you will not need to repeat the first four items above (Title through Procedure). However, carefully state any departures from the printed directions either in the use of materials or in the procedures followed. The most important part of your report obviously is that which relates to the data, the discussion of results, and the conclusions you draw.

Always write in clear and simple language, using words as precisely as you can. Avoid ambiguity; that is, don't let your language have vague or double meanings. When you can express data quantitatively, do so as precisely as possible, using the metric system. Avoid the appearance of greater precision than you have obtained. To state that something weighed 1.002 g when your scales weigh no more precisely than 1.0 g is unscientific. In averaging results, round off only to the same position right or left of the decimal point as the measurements taken. To carry the average to several decimal places beyond those taken in measurements leads to a false impression of accuracy. This again is unscientific.

Drawings should be outline drawings made with a firm pencil and continuous lines. Label structures neatly placing all names horizontally on the right-hand side of the drawing. Label lines running to the structures should not cross. Be sure to give the drawing a title, preferably placed below it. Avoid shading (except by stippling) since it usually obscures more than it reveals. In general, your drawings should look like the diagrams in your laboratory guide.

Graphs of quantitative data are important in scientific communication. Use them whenever you can and, when you are graphing results, take pains to plot the points accurately and to connect them with smooth lines. Label the quantities plotted on the vertical axis (ordinate) and horizontal axis (abscissa) and provide the entire graph with a concise title. Your complete reports should be kept in a looseleaf notebook for future reference.

Always remember that communication is an important part of science. Discoveries about nature become a part of science only when they are made known by the discoverer to others—when they are published to become the common property of mankind. In publishing scientific work, an investigator must state his procedures and results in such a way that another person can repeat the procedures exactly. You must state the exact material used (in biology this means the species of plant or animal or microorganism used and the genetic strain, if it is known). You must give every step of the procedure, as well as the analysis and interpretation of the results. Only by schooling yourself in these methods can you learn why science is so successful in achieving results and in advancing a common effort that is truly international in scope. Scientists must be free to communicate, and they must know how to communicate. Be proud of your developing capacity in these respects.

TEACHER'S MANUAL ORGANIZATION

The numerical organization of exercises in the *Teacher's Manual* follows that of the *Student Laboratory Guide*. Each laboratory exercise is keyed to revelant material in the textbook, and the time involved in performing each exercise is given. The suggested times may vary, of course.

Materials

The list of materials for each exercise is given in the *Student Laboratory Guide*. The amount of materials necessary will depend upon whether students work as individuals or in teams of various sizes.

Many teachers have found it advantageous to prepare student kits in convenient containers, such as cigar boxes. The student kits should include basic items to be used throughout the course, such as forceps, scissors, two dissecting needles, four Barnes dropping bottles (for distilled water, Ringer's solution, stains as required [iodine and methylene blue suggested]), about five microscope slides and cover glasses (square cover glasses preferred), lens paper, about four medicine-dropper pipettes, a 15-cm plastic ruler, a glass tumbler or other vessel to hold test tubes, paper towels, and two single edge razor blades suitably protected. The authors suggest about one kit per microscope. In most schools this would mean two to four students per kit. It is not absolutely necessary for each student to have an individual kit. At the other extreme it is undesirable for more than four students to share a kit or a microscope unless absolutely necessary. Of course, a few extra kits should be prepared, to anticipate loss and breakage.

In the laboratory the students will use a great number of chemicals and solutions. Where special danger exists, as in handling acids and alkalies, cautions are provided for both the student and teacher. However, it is essential to recognize that *all chemicals should be handled with care*. They should all be treated as if they are poison, for this is merely a relative term. In certain quantities even water, aspirin, and table salt will act as poisons. Therefore, treating all chemicals and solutions with caution and care, regardless of whether they are labeled as poison, is good laboratory practice.

Purpose

The purpose of the exercise will be similar and occasionally identical to that given in the *Student Laboratory Guide*, depending on whether or not the teacher should have additional objectives in mind for the exercise.

Special Instructions

The section devoted to special instructions points out particular features of the exercise that should be brought to your attention immediately.

These features include not only procedural problems, but also emphases and cautions together with the requirements for advanced preparation.

Techniques

The techniques section explains the technical aspects of the exercise and offers hints and suggestions as to the best ways of handling the exercise.

Answers to Questions

The answers to the questions contained in the *Student Laboratory Guide* are given for each numbered question in each exercise. These answers are often more complete and complex than those expected from the student and are primarily provided for the information of the teacher in interpreting the student's answer. By no means is it intended that the student give answers as detailed and complete as some of these may be.

Suggested Readings

The suggested readings are particularly directed to students who wish to go beyond the scope of the exercise. The answers to many of the student questions which will arise during the course of the exercise will be found in the suggested readings. It would be well to check the biology library or the school library to see that many of these references will be available.

Films

The final section on films is provided for the use of the teacher. In some cases films are essential, in some cases advisable, and in others completely optional. The philosophy of using films is found, along with the abbreviations used in the film list, at the end of this *Teacher's Manual.*

Recognizing that teachers have full and busy schedules, the *Teacher's Manual* is designed to facilitate, wherever possible, teaching the laboratory portion of *Biological Science: An Inquiry into Life.* Your comments and questions will be gratefully received and answered by the undersigned so that the future editions will contain the combined wisdom of teachers who have participated since 1960 in this endeavor.

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UNITY

Cells

The laboratory is an integral part of the BSCS biology program, and therefore, the proper inauguration of laboratory work is essential to future successes. Because it is difficult in the first weeks of school to present laboratory work which requires a great deal of preparation of materials, supplies, and space, the exercises designed for the first two chapters are supplementary and can be done as teacher demonstrations. As exercises in the scientific approach to a problem they serve to introduce the student to experimentation in a relatively simple fashion. Exercise 2-1 is particularly attractive to students and gives the teacher an opportunity to introduce meaningful discussions of the problems of experimental method and abiogenesis.

The student becomes acquainted with the tools of the biologist in working with the compound microscope and making microscopic measurements. These two exercises should be done thoroughly and with great care in order to make certain that the student has mastered the principles therein. With the development of the scientific enterprise and the mastery of the basic tools, the student then begins the introduction to cells, first from a historical standpoint repeating the classic work of Robert Hooke, and then by observing living plant cells and other single-celled organisms. A culmination of the work on cells is the process of having the student develop his own concept of generalized cell structure from his laboratory observations.

The study of the cell naturally leads the student to desire to know more about these units of life. With less emphasis on chemistry and more emphasis on the biology involved, the student is led through a series of exercises encompassing the activities of cells. In these exercises chemistry is treated as a basic part of the biological process being observed and not as an isolated abstraction for the student. The completion of these exercises will leave the student with an understanding of the importance of chemistry in cellular action and the various factors which influence cellular reactions.

From the understanding of the single cell the student is prepared for the later study of multicellular organisms and, for this purpose, a comprehensive exercise on mitosis is designed to explain the process of cell duplication. This introduces reproduction in the laboratory. Future discussions of reproduction will be based upon this introduction. By using nitrogen-fixing bacteria and a leguminous plant as an illustration, the final exercise of this section is intended to impress upon the student the interrelationship of organisms and their dependence one on another.

In that it lays the groundwork for all future laboratory activities of the course and presents the basic materials relative to the scientific enterprise, the tools of science, and the cellular organization of living organisms, this unit can be considered to be the most important unit of laboratory work to be attempted in the course. Additional effort put into imaginative presentation and mastery of these early exercises will be rewarded by accelerated appreciation of subsequent work on the part of the student.

STATE INSTITUTE OF EDUCATION ★ MANIPUR ★

A BIOLOGICAL PROBLEM—MALARIA

EXERCISE

1-1

TIME: One laboratory period.

MATERIALS: See page 3 in the *Student Laboratory Guide.*

The student should read Chapter 1 of the textbook before observing the laboratory demonstration.

■ **The purpose of this exercise is to reinforce the student's understanding of the nature of the scientific process as exemplified by research on malaria.**

SPECIAL INSTRUCTIONS

This exercise is designed as a demonstration to answer some of the questions which will probably arise concerning the details of the malarial parasite's life cycle. However, caution must be exercised in order not to become too involved in the details of malaria *per se*. We recommend that references containing detailed stages of malaria (merozoite, sporozoite, etc.) not be used. Notice that the diagram of the life cycle of malaria in the *Student Laboratory Guide* is concerned only with broad aspects.

TECHNIQUES

In order to emphasize the methodology involved you may wish to use "Invitations to Enquiry," Nos. 10 and 18 in the *Biology Teachers' Handbook.* These invitations deal with environmental factors and disease, and disease and treatment. They involve an investigation of simple inquiry, Invitation 10 concentrating on the idea of hypothesis, and Invitation 18 covering unit causes.

Demonstration slides of various stages in the malarial life cycle viewed under the compound microscope will impress the student with the difficulty of seeing the organisms with which people such as Ross were working. It will also serve to reinforce the idea of the absolute necessity of good microscopes in order to complete the life cycle story. Here is a chance to stress the idea of the importance of equipment for the advancement of certain investigations at critical stages.

You may wish to project slides of malarial life cycle stages. Microscope slides cannot be projected easily, because oil immersion is necessary. However, Kodachromes of these stages are distributed by the Clay-Adams Co. in their "Medichrome" Series MT entitled "Tropical Diseases." In this series, Kodachromes of *Plasmodium vivax* are numbered MT45, 45A, 45B, and 58.

Charts, pictures, and diagrams can be used to reinforce the cyclical nature of the malarial parasite's life and the general problems presented by the disease.

ANSWERS TO QUESTIONS

1. Parasites in the blood are so tiny that they can be seen only with a good microscope.
2. (a) The individual originally bitten was not infected with malaria; or (b) the person was bitten by a mosquito other than an *Anopheles*; or (c) the mosquito bit the uninfected individual before the life cycle was completed in the mosquito. Thus, there was inadequate incubation time between the first and second bite.
3. None of these would cause malaria. It is caused only by the bite of an infected *Anopheles* mosquito.
4. Rupture of all red blood cells would cause the death of the infected person. However, all the red blood cells are never invaded.
5. The life cycle of *Plasmodium* would be broken, as the parasites would not get from the mosquito's intestine to its mouthparts and hence in a position where they could be injected into the bloodstream of the host.
6. The cycle is most readily broken in the mosquito stage, either by killing the mosquitoes or by screening to keep them away from man.
7. No symptoms would develop in the bitten men.
8. No. The sample is too small. It would need to be tried on a larger group. Also, there are no controls bitten by the mosquitoes but not receiving the drug.
9. Controls.
10. It not only removes the symptoms temporarily but seems to prevent the disease and to control symptoms over a long period of time.
11. An adequate public health program of mosquito control and antimalarial measures has been in effect in the United States for a considerable period of time.
12. The scientific methods are similar in principle, if not identical in detail to those described for malaria.

SUGGESTED READINGS

Books

De Kruif, P. 1959. *Microbe Hunters.* Pocketbooks, New York

Dulos, R. 1962. *Mirage of Health.* Doubleday, New York

Articles

Alvarado, C. A. and L. J. Bruce-Chwatt. 1962. "Malaria." *Sci. Am.* **206** [5] 86

FILMS

Chills and Fever: Why?, 1947, (fs, sd, c, 53 fr, 13 min), r, USPH

Erythrocytic Stages of Plasmodium vivax, 1954, (sd, b&w, 4 min), r, USPH

The Life Cycle of a Malaria Parasite, 1948, (fs, sd, c, 93 fr, 13 min), r, USPH
Vivax *Malaria*, 1948, (fs, sd, c, 76 fr, 17 min), r, USPH

The following films will provide interesting points of departure for classroom discussions of the methodology of scientific investigation. Each deals with a particular disease which parallels the research on malaria in investigatory process.

Wheat Rust, 1959, (sd, b&w or c, 15 min), p or r, EBF
Man Against Microbe, 1933, (sd, b&w, 11 min), free loan, Metropolitan
Scientific Methods, 1954, (sd, b&w or c, 12 min), p or r, EBF
Story of Dr. Jenner, 1939, (sd, b&w, 10 min), MGM
Yellow Jack, 1951, (sd, b&w, 29 min), r, TFC
The Tragic Hour of Dr. Semmelweis, 1955, (sd, b&w, 28 min), YAF
Born in the White House, 1953, (sd, b&w, 26 min), National Foundation

EARLY EXPERIMENTS IN SPONTANEOUS GENERATION

EXERCISE 2-1

TIME: One laboratory period.

MATERIALS: See page 5 in the *Student Laboratory Guide*.

The students should read Chapter 2 of the textbook dealing with the work of Spallanzani and Pasteur in relation to spontaneous generation before observing the laboratory demonstration.

■ **The purpose of this exercise is to visualize the work of Spallanzani and Pasteur.**

SPECIAL INSTRUCTIONS

It is time-consuming to make special tubing and to bore corks the first time, but once this is done all materials can be kept for reuse in succeeding years. To make preparation easier, test tubes may be substituted for flasks and correspondingly smaller quantities of broth used. This may make it possible for several groups of students or even the entire class to perform this exercise as a laboratory experiment.

How this material will be used will depend on the amount of time and equipment available. It can be done as a teacher demonstration; it can be assigned as a special project to be performed by a single student or a small group of students; or it can be performed by the entire class, either by individuals or teams of students.

As there are seven experiments in this exercise, it has been found practical to use fourteen teams of students, two teams assigned to each part of the exercise and serving as checks on one another.

In such team-directed activity it is necessary to pool the results from the various teams and have a class discussion period so that the experiment can be seen in its entirety.

The exercise can be performed in a single period or its results can be demonstrated in a single period. However, the exercise loses its excitement if the student sees only the beginning or only the end. Student participation and involvement, together with periodic observations of the results, make this exercise far more meaningful than its use as a single period demonstration.

TECHNIQUES

The temperature of the autoclave or pressure cooker at 15 lbs pressure is 120° C (Celsius). Flasks can be dry sterilized in an oven at 175° C for an hour.

If cork stoppers are to be sealed to the flasks with paraffin, the flasks should not be too hot in order to keep the paraffin from melting and running down the sides.

Cover the unstoppered flasks with screen or gauze to keep out insects.

Caution the students not to handle or open the flasks during demonstration.

Failures are due to inadequate procedures. Spore formers may not have been killed by boiling for example.

Put 70 ml of the broth or bouillon into each flask.

Treat the flasks as follows:

Flask 1: Overall control

Plug with cork stopper and seal the cork to the flask with warm wax or paraffin. Do not heat.

Flask 2: Spallanzani's control

Boil gently for 15 minutes. Leave open.

Flask 3: Spallanzani's experiment

Boil gently for 15 minutes. After heating, close with a cork. Seal with wax or paraffin.

Flask 4: Pasteur's control

Heat in the pressure cooker or autoclave for 15 minutes at 15 lbs pressure. Leave unplugged.

Flask 5: Modified Pasteur's control

Plug with a cork stopper through which an open, straight glass tube has been inserted. Heat in the pressure cooker or autoclave as for Flask 4. Then seal the cork with wax or paraffin.

Flask 6: Pasteur's first experiment

Plug with a cork stopper through which the C-shaped glass tube has been inserted. Heat in pressure cooker or autoclave as for Flask 4. To seal the exposed end of the C-tube, heat gently and plug with wax or paraffin.

Also seal the cork to the flask.

Flask 7: Pasteur's final experiment

Plug with a cork stopper through which the S-shaped tube has been inserted. Heat in the pressure cooker or autoclave as for Flask 4. Seal the cork to the flask with warm wax or paraffin.

Put all the flasks on a laboratory table (not in direct sunlight or over a radiator). The students should look for changes in the flasks, at first from day to day, later on from week to week. When any changes in the appearance of the flasks occur, they should be recorded and any odor present in those which are open should be noted. No corks should be removed, however.

Observations are to be recorded on the accompanying chart (Figure 2-1-1) in the *Student Laboratory Guide* or on a similar form. Changes can be shown by shading the contents of the flask and stating in the spaces under the flasks the times when changes appear.

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ANSWERS TO QUESTIONS

1. Typical results of the experiment are as follows:
   Flask 1 will become turbid within a day or two.
   Flask 2 will become turbid, but usually after a longer time than Flask 1.
   Flask 3 will probably take still longer to become turbid. Although many of the microorganisms will have been killed by heating, the resistant spores of bacteria will survive.
   Flask 4 will show growth after some microorganisms have entered from the air.
   Flask 5 with its small opening may not show growth for several days or even a week or two but if kept long enough, growth will occur.
   Flasks 6 and 7 should remain free of microorganisms. Flask 6, completely sealed, does not allow access of air to the contents. Flask 7 does, thereby removing the objection of the believers in spontaneous generation that air which is vital to life has been excluded. The design of the S-tube, roughly comparable to that of Pasteur's flask, prevents the entrance of microorganisms unless air is blown in.
   Bacterial growth will most likely appear first and molds may appear later.
2. In view of the observations of the flasks, the student should deduce that the microorganisms in the nutrient broth have come from the air.
3. To act as controls and check on the ability of microorganisms to grow in the boiled broth.
4. The S-shaped tube allows air to enter the flask but prevents microorganisms from entering.
5. Pasteur's experiment was more successful than Spallanzani's because it answered the objections of the abiogenists, namely, that air had been excluded from the flasks.
6. An abiogenist might say that the vital force in the air had been ruined and new, vital air prevented from entering.

7. If the conclusion is drawn that microorganisms come from the air, practical aspects of this experiment would involve boiling to kill microorganisms and sealing to keep further microorganisms from entering. This is done in food canning and other similar processes.

SUGGESTED READINGS

Books

Brock, T. (ed.) 1961. *Milestones in Microbiology, Part I, Spontaneous Generation and Fermentation.* Prentice-Hall, Englewood Cliffs, N.J.

Conant, J. B. (ed.) 1953. *Harvard Case Histories in Science: Pasteur's and Tyndall's Study of Spontaneous Generation.* Harvard Univ. Press, Cambridge, Mass.

Gabriel, M. and S. Fogel (eds.) 1955. *Great Experiments in Biology, Part 3, Microbiology.* Prentice-Hall, Englewood Cliffs, N.J.

Schwartz, G. and P. Bishop (eds.) 1958. *Moments of Discovery, Vol. 1, Origin of Life.* Basic Books, New York.

FILMS

Microbiology, Part II: *The Very Small,* No. 1, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Microbiology, Part II: *The Germ Theory of Disease,* No. 10, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Man Against Microbe, 1933, (sd, b&w, 11 min) free loan, Metropolitan

USING THE COMPOUND MICROSCOPE

EXERCISE

3-1

TIME: One laboratory period.

MATERIALS: See page 7 in the *Student Laboratory Guide.*

■ **The purpose of this exercise is to acquaint the student with what may be the single most important tool in his biology laboratory.**

SPECIAL INSTRUCTIONS

As a prerequisite to learning to use the microscope and also to stimulate interest, you may wish the students to read the section of the textbook in Chapter 3 on the invention of and early work with the microscope.

Part E may be assigned as homework. The use of a tumbler of water is the only manipulation in E and this can be done at home. Question 7 can be answered outside class and turned in as a homework exercise.

The student will be using the microscope for considerable portions of the laboratory periods in this course. Therefore, it is essential that he become thoroughly grounded in its use. Careful attention to this exercise will alleviate much potential difficulty in future laboratory work.

The microscopes should be numbered and assigned to specific students each of whom will be responsible for the care of his microscope. Throughout the year it will be essential to check that the students are using and handling the microscope properly.

They should be instructed in cleaning the lenses of the microscope properly because the soft glass of the lenses is easily scratched. They should be wiped with one-way motion across the diameter of the lens. Circular motion may scratch the lens by grinding dust around on it.

Although plastic cover glasses do not break as easily as glass ones, they are scratched more easily. Alcohol cleans cover glasses and slides better than water alone.

TECHNIQUES

Parfocal Determination. It is suggested that you determine whether or not the microscopes you use are parfocal. If a microscope is parfocal the object remains in focus regardless of which objective is being used. Microscopes can be purchased already parfocalled or there are techniques for making nonparfocal microscopes parfocal. The advantage is, of course, that the specimen remains in focus at each change of objective. In cases where microscopes are already parfocal it is not necessary to raise the body tube before switching to high power. In microscopes that are not parfocal, the procedure of raising the body tube before switching to high power should always be followed. If this technique is not followed and if not done slowly and through a short distance only, damage to the slide and objective can result.

Centering the Specimen. Since the specimen does not always remain centered when the objective is changed, each student should check his microscope for its centering problems. Ask him to make a circle representing a low-power field on a piece of paper. Then place an "X" in the center of the circle where the specimen is located under low power. Now have him locate the specimen under high power without moving the slide. Where is the specimen in the field? Have the student place an "O" in the circle where the center of the specimen is seen under high power. If the "X" and "O" are in the same place in the circle the objectives are centered. If not, in future work the student should move his specimen to the "O" position while still under low power *before* switching to high power. Then when he switches to high power, the specimen will be centered properly for observation.

Using Both Eyes. The student should be cautioned to use the microscope with both eyes open in order to avoid undue fatigue and possible eye strain. If the student has initial difficulty in achieving this, have him cover one eye with his hand (the right eye if right-handed, the left eye if left-handed) until he sees the field well. Then if he slowly removes his hand while looking through the ocular, he should be able to retain the microscope image.

Fine Adjustment. Special attention should be paid to instructing the student in the use of the fine adjustment. If the object is brought into focus as much as possible with the coarse adjustment, it should not be necessary to turn the fine adjustment knob more than a turn or two in any direction. The fine adjustment knob can be turned only several revolutions in any one direction before reaching its stopping point. If the stopping point is reached before the fine focusing is complete, raise the body tube with the coarse adjustment and then turn the fine adjustment back to about the middle of its range to restore it to use. The microscope can then be focused as before, starting with the coarse adjustment.

Review the *functions* of the various parts of a microscope.

ANSWERS TO QUESTIONS

1. The letter "e" is not only inverted (upside down) but also reversed, right to left, and left to right.
2. As the student moves the slide from right to left the image moves from left to right.
3. As the student moves the slide away from him the letter "e" appears to move toward him.
4. It may change the position of the image depending upon the individual microscope.
5. The field of view is smaller in that a smaller part of the letter "e" is seen enlarged.
6. The illumination is less under high power and the diaphragm should be opened to compensate for the reduced illumination.
7. 200 mμ

SUGGESTED READINGS

Books

Needham, G. H. 1958. *Practical Use of the Microscope.* Charles C. Thomas, Springfield, Ill.

FILMS

Compound Microscope, 1953, (sd, b&w or c, 20 min), p, Bausch & Lomb
Microscope and Its Use, (sd, b&w, 10 min), YAF(McGraw-Hill)
Compound Microscope, 1955, (fs, b&w, 24 fr), VEC

MICROSCOPIC MEASUREMENTS

EXERCISE 3-2

TIME: One laboratory period.

MATERIALS: See page 13 in the *Student Laboratory Guide.*

■ **The purpose of the exercise is to acquaint the student with the use of the metric system and the application of metric measurements to microscopy.**

SPECIAL INSTRUCTIONS

It will be advantageous to determine students' previous contacts with the metric system. Depending on their backgrounds, a review session may be necessary to bring the students' proficiency in the metric system up to the stage where this exercise can be encompassed in the period allowed.

"Invitations to Enquiry," No. 5 in the *Biology Teachers' Handbook,* which introduces the concept of experimental error, may prove valuable.

Reinforce understanding of the metric system by having students convert English units of measurement to metric units and vice versa. Ask them to calculate magnifications from given lens combinations.

ANSWERS TO QUESTIONS

1. 100 cm.
2. 1000 mm.
3. 39.37 inches.
4. 2.54 cm.
5. 25.4 mm.
6. 10 mm.
7. Variable depending on size of table.
8. To be determined by the student.
9. $4 \times 10 = 40$; $10 \times 10 = 100$; etc.
10. $40 \times 10 = 400$; $44 \times 10 = 440$; etc.
11. These measurements are approximations. 3.5× objective = 3.6 mm, or 3600 μ; 5× objective = 3.0 mm or 3000 μ; 10× objective = 1.5 mm or 1500 μ; 43× objective = 0.25 mm, or 250 μ.
12. Variable with objective and ocular combination used. Divide the magnification of the high-power objective by the magnification of the low-power.
13. Repeat in microns the answer to Question 11.
14. Divide the diameter of the low-power field in microns (13 above) by the ratio (12 above).
15. Measure the largest diameter of the cell (250 μ is near the top of the range for most algae) and divide this into the length of drawing in millimeters.

SUGGESTED READINGS

Books

Gage, S. H. 1941. *The Microscope.* 17th ed. Comstock, Ithaca, N.Y.

Gray, P. 1954. *The Microtomist's Formulary and Guide.* Blakiston (McGraw-Hill), New York

Guyer, M. 1953. *Animal Micrology.* 5th ed. University of Chicago Press, Chicago

Lee, A. B. 1950. *Microtomist's Vade-Mecum.* 11th ed. J. Gattenby and H. Beams (eds.) Blakiston (McGraw-Hill), New York

FILMS

Weighing Techniques, 1961, (sd, c, 8 min), p or r, Thorne
Measuring Techniques, 1961, (sd, c, 14 min), p or r, Thorne
Measuring Short Distances, 1959, (sd, b&w, 20 min), r, MLA

CELLS AS ROBERT HOOKE FIRST SAW THEM

EXERCISE
3-3

TIME: One laboratory period.

MATERIALS: See page 17 in the *Student Laboratory Guide.*

■ **The purpose of the exercise is to acquaint the student with a simple, obvious cell; to correlate structure and function; and to repeat one of the classical biological observations of all time.**

SPECIAL INSTRUCTIONS

Students cutting freehand sections in the manner shown in the *Student Laboratory Guide* must be cautioned about the danger of cutting themselves. Use of large bottle corks (No. 10) will minimize the danger.

Stress the fact that very *thin* sections are critical for this exercise.

Emphasize to the students that they are repeating one of the great classical experiments which led to the coinage of the word *cell* and which eventually led to the knowledge that plants and animals are made up of cells.

TECHNIQUES

A very simple device for cutting cork without endangering fingers can be made by taking a large hexagonal nut into which a precut piece of cork can be threaded. By the simple process of twisting the cork slightly, one

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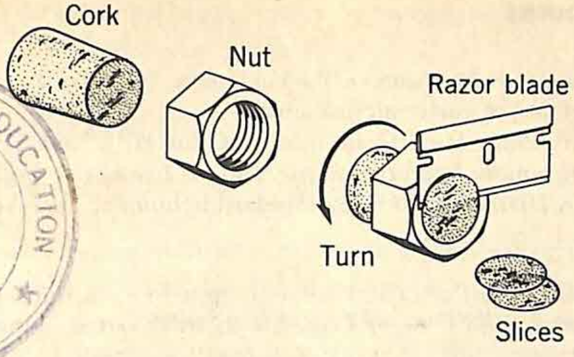

Figure 3-3-1 **Nut method of cutting cork sections.**

end will project from the nut. If you then run a razor blade along the surface of the nut a thin slice of cork will be removed (Figure 3-3-1, shown above). With a little practice, cork sections of satisfactory thinness and evenness can thus be produced.

Another source of ready-cut, thin cork is the cork-tipped filter on a cigarette. Filters can be removed from cigarettes and soaked until the thin layer of cork becomes detached. This thin cork layer provides an admirable ready-made slice for the purposes of this exercise. Note that many "cork" tips are really paper. Only a few brands use real cork.

A minimum number of air bubbles will be trapped between the cork, the slide, and the cover glass if a drop of water is placed on the slide first, then the piece of cork, followed by a second drop of water on top of the cork before putting on the cover glass.

ANSWERS TO QUESTIONS

1. Protection from blows or abrasions (because of compressibility), and prevention of water loss (because of imperviousness). The walls of cork cells are impregnated with a fatty substance called suberin. Because of this the cork tissue forms an effective water-proofing layer on the trunk and branches of the tree and prevents excessive water loss from the plant.
2. They are not all of the same shape. The shape depends on the cut. They may vary from rectangular to almost round.
3. Rectangular. Interpretation will vary depending on the direction and angle of the student's cut section.
4. No structures can be seen.
5. No, the cells fit closely together.
6. The impervious cell walls.
7. There is air in the cells.
8. Nothing that can be seen in these observations gives evidence of life or former life of these cells unless the student already knows that the walls and their chemical composition are formed only by living cells.

SUGGESTED READINGS

Books

De Kruif, P. 1959. *Microbe Hunters.* Pocket Books, New York

Short biographies of early microscopists.

Johansen, D. 1940. *Plant Microtechnique.* McGraw-Hill, New York

Sass, J. E. 1956. *Botanical Microtechnique.* 2nd ed. Iowa State College, Ames, Iowa

Singer, C. 1950. *A History of Biology.* Abelard-Schuman, New York

FILMS

The Cell—Structural Unit of Life, 1949, (sd, b&w or c, 11 min), p, Coronet

Cell Biology, Part I: *The Unit of Life,* No. 2, 1960, (sd, c, 30 min), p or r, AIBS (McGraw-Hill)

LIVING PLANT CELLS:
Onion Epidermis

EXERCISE
3-4

TIME: One laboratory period.

MATERIALS: See page 19 in the *Student Laboratory Guide.*

■ **The purpose of the exercise is to introduce the student to living cells and their basic structures.**

SPECIAL INSTRUCTIONS

Until the student becomes versed in preparing wet mounts it is advisable to have the mounts prepared on the desk rather than on the microscope stage. This will avoid spilling water and stains on the microscope stage and possible damage to parts such as the condenser.

Onion epidermis can cause great difficulty when folded sections are used so that the student looks at wrinkled specimens two cells thick with air bubbles trapped between them. Therefore, stress the necessity of obtaining unwrinkled specimens.

TECHNIQUES

The water of the wet mount should come to the edge of the cover glass in order to allow the stain to be drawn under. Too much water, however, allows the liquid to spill over the edge of the slide. If the underside of the slide should become wet, the student should dry it on toweling before placing it on the stage of the microscope.

Do not microproject. Let the student discover at this stage.

Do not tilt the microscope stage with wet mounts, as the liquid may run off the slide into the microscope parts.

ANSWERS TO QUESTIONS

1. Elongated rectangles, irregular in outline.
2. Yes, but thinner than those of cork.
3. Cytoplasmic streaming should indicate that the cell is alive.
4. Round when viewed from above but oval when seen from the side.
5. In the cytoplasm next to the cell wall. If it seems to be in the center it is really against the top or bottom of the cell which can be determined by focusing up and down.
6. Because different parts of the cell are brought into focus as the body tube is moved up and down. Stress the three-dimensional nature of cells.
7. No, it is concentrated around the edges of the cell wall.
8. It has a granular appearance.
9. The stained cytoplasm is not moving.
10. The staining and handling has caused the cell to die.
11. In the center of the cell in the vacuole.
12. The cork has a thicker cell wall and is less flattened. No cytoplasm or nucleus is visible in the cork cell.
13. Both have cell walls and both serve a protective function.

SUGGESTED READINGS

Books

Butler, J. A. V. 1959. *Inside the Living Cell.* Basic Books, New York
Swanson, C. P. 1960. *The Cell.* Prentice-Hall, Englewood Cliffs, N.J.

FILMS

The Cell—Structural Unit of Life, 1949, (sd, b&w or c, 11 min), p, Coronet
Cell Biology, Part I: *What Is a Cell* (Cell Morphology) No. 3, 1960, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)

LIVING PLANT CELLS WITH CHLOROPLASTS

EXERCISE 3-5

TIME: One laboratory period.

MATERIALS: See page 21 in the *Student Laboratory Guide.*

■ **The purpose of this exercise is to introduce the student to a given plant cell and its pigment-containing chloroplasts.**

SPECIAL INSTRUCTIONS

Living *Anacharis* (elodea) can be obtained from a local pet shop or from a biological supply house. It can be planted and kept growing in a well-aerated aquarium provided the snail population is not excessive. Getting the cytoplasm with the contained chloroplasts to show movement may present some difficulty in this exercise. The chances are good that the students will see cytoplasmic streaming (cyclosis) if fresh material is used and if young leaves near the tip are picked. Illuminating *Anacharis* for about 12 hours before it is used also enhances the chances that the students will see cytoplasmic streaming. Gentle warming also increases cyclosis but care must be taken not to get the material *too* warm! Simply breathing on the elodea leaf on the slide usually accomplishes increase of cyclosis.

ANSWERS TO QUESTIONS

1. Cells without chloroplasts do not manufacture food. Those with chloroplasts do so by the process of photosynthesis.
2. Chloroplasts are disk-shaped. This can be determined by looking at several chloroplasts under high power at different angles in order to appreciate their flattened three-dimensional character.
3. Chloroplasts are located in the cytoplasm which, in turn, is next to the cell wall. They can be seen, by careful focusing, along the walls of the cell. If they appear to be in the center of the cell the chances are the student is looking at either the top or the bottom of the cell.
4. Chloroplasts are passive objects. They are carried around the cell by the moving cytoplasm. No one knows what makes the cytoplasm stream. This is one of the most interesting problems in biology.

SUGGESTED READINGS

Books

Hill, R. and C. P. Whittingham. 1955. *Photosynthesis.* Wiley, New York

Articles

Frank, S. 1956. "Carotenoids." *Sci. Am.* **194** [1] 80

FILMS

Multicellular Plants, Part III: *Chlorophyll*, No. 4, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)

VARIETIES OF ANIMAL CELLS

EXERCISE 3-6

TIME: One laboratory period.

MATERIALS: See page 23 in the *Student Laboratory Guide.*

■ **The purpose of the exercise is to acquaint the student with various types of animal cells and their adaptations for the functions they perform; secondly, to allow the student to compare animal cells with plant cells seen previously.**

SPECIAL INSTRUCTIONS

Students should be cautioned to scrape the inside lining of the cheek *very gently*. Harsh scraping may result in bleeding and is unnecessary for the purposes of this exercise. A sterile tongue depressor may be used in place of a toothpick.

The success of this exercise depends upon the availability of the frog cells as students are ready for them. Therefore, the suspensions of cells and pieces of tissue should not be prepared much in advance of the lesson but just prior to it so that the materials will be as fresh as possible.

TECHNIQUES

Preliminary preparations require one or two frogs which have been etherized or pithed and dissected, pipettes, a dissecting set, and three small beakers or vials containing Ringer's solution.

Label the three containers SKIN CELLS, BLOOD CELLS, and SPERM CELLS.

Blood. With a medicine dropper suck up the blood from a slit in the heart or a large blood vessel, or if the frog is bleeding, simply flush the area with Ringer's solution and suck up the suspension. Place it in the dish labeled BLOOD.

Skin. You may use the external layer of skin that has been sloughed in the water in which the frog was kept, or, with a sharp scalpel, scrape off some of the skin of the frog and deposit the scraped material in the dish labeled SKIN.

Sperm. Males of *Rana pipiens* can be distinguished from females by the enlarged thumb pad on the "hand" (Figure 3-6-1). In bullfrogs males have a yellow coloration under the "chin" and a larger tympanic membrane than females.

Place the frog testes in a dish containing 10 ml of Ringer's solution. Using a scalpel or razor blade, cut the testes into small pieces, mincing them thoroughly. Forceps or dissecting needles may be used for this mincing process.

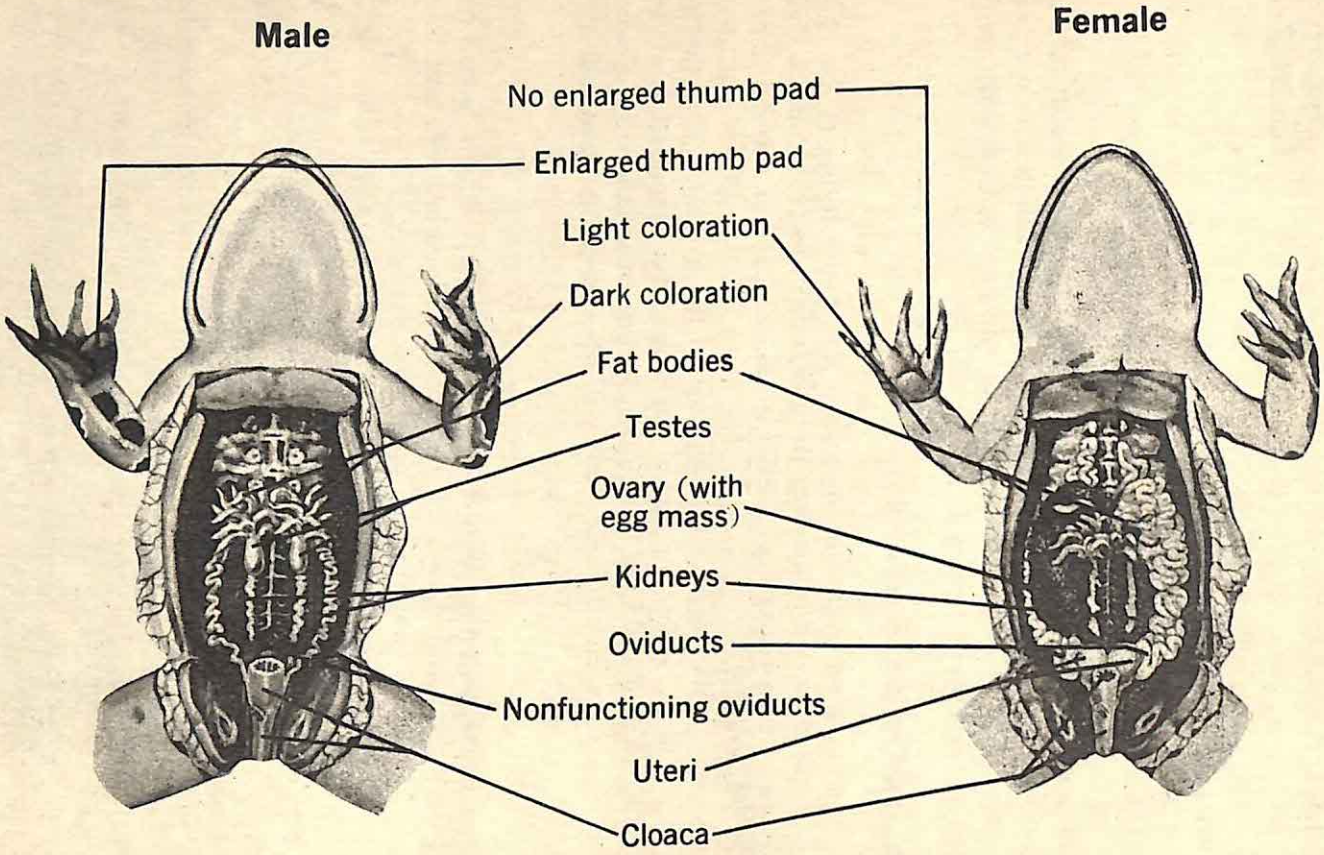

Figure 3-6-1 **Male and female frog anatomy.**

For the better students you may wish also at this time to use the following additional frog tissues.

Ciliated Epithelium. Cut off the lower jaw; then cut out small pieces of the lining of the upper jaw from the region of the eye bulges to the throat. Place these in Ringer's solution in a small dish. Observe with the high power of the stereoscopic dissecting microscope, as well as with the compound microscope.

Muscle. Remove the skin from the hind legs. Dissect out the large muscles from the shanks and cut them into small pieces. Place the pieces in a dish of Ringer's solution.

Nervous Tissue. Dissect out the spinal cord and cut it into small pieces, placing the pieces in a dish of Ringer's solution.

ANSWERS TO QUESTIONS

1. Animal epithelial cells lack the rigid cell walls of plant cells.
2. The flatness of the epithelial cells allows each cell to cover a relatively large area of the lining of the mouth.
3. To be determined by the student. The ratio is variable but may commonly be 1:10. The nucleus is not a sphere nor is the cell of equal diameter so latitude must be given for selection of measurements.
4. Yes, a relatively large oval nucleus.
5. Yes, a relatively smaller nucleus than that of the blood cell.
6. It is a flattened, irregularly polygonal cell.
7. It may be described as a small commalike object or a tiny tadpole-shaped cell with a relatively large head and a slender tail.
8. Sperm cells should still be alive and should be seen to move by means of the lashing back and forth of their tails.

SUGGESTED READINGS

Books

De Robertis, E. D. P., W. W. Nowinski, and F. A. Saez. 1960. *General Cytology.* W. B. Saunders, Philadelphia

Pantin, C. F. A. 1946. *Notes on Microscopical Techniques for Zoologists.* Cambridge Univ. Press, New York

Swanson, C. P. 1960. *The Cell.* Prentice-Hall, New York

Articles

Upjohn Co. 1958. "Scope Monograph on Cytology: The Cell." Kalamazoo, Mich.

FILMS

The Cell—Structural Unit of Life, 1949, (sd, b&w or c, 11 min), p, Coronet

Cells and Their Functions, 1950, (sd, b&w, 14 min), p or r, Contemporary

LIVING SINGLE-CELLED ORGANISMS

EXERCISE

3-7

TIME: One or two laboratory periods.

MATERIALS: See page 25 in the *Student Laboratory Guide.*

■ **The purpose of the exercise is to acquaint the student with a variety of free-living single-celled organisms in their natural state, to observe the structure and function of a single-celled animal in its finer detail, and to see a protozoan which is not free-living and which is dependent on another animal for its environment.**

SPECIAL INSTRUCTIONS

Involve the students in this "animal hunt" by requesting that they bring samples of water to the laboratory for the purposes of the exercise. Students may be able to bring water from creeks, ponds, rivers, lakes, puddles, or swamps, etc. Each should be labeled as to its source so the students can contrast the communities of species in various water samples.

Check the cultures brought in beforehand so that you will have some idea of the variety of single-celled organisms contained in them and some idea of which cultures are rich and which poor in organisms.

TECHNIQUES

The methyl cellulose used to slow down protozoa is slowly soluble in water and therefore must be made up some time in advance. Methyl cellulose tends to distort the shape of microorganisms as they move through the thick solution. You may want to use it for Parts A and C as well as B, but the students should see normal movements before it is used.

Commercially available transparent starch can be used as a substitute for methyl cellulose. Also, removing some of the water beneath the cover glass by absorption with paper toweling will slow down the movement. In the earlier parts of the exercise it is advisable to caution the student about evaporation of the water which, of course, will destroy the organisms on the slide.

If pond water is obtained sufficiently far in advance, it will be possible for the teacher to prepare pure cultures from any selected one-celled organism present. This can be accomplished easily with a stereoscopic microscope and with a pipette prepared as follows. Heat the end of a pipette over a Bunsen burner and draw it out to capillary dimensions. Break off the excess glass. Place the index finger over the large end of the pipette before placing the capillary end in the water. When the capillary end is very close to the organism to be captured, remove the index finger. You will discover that the organism is quickly sucked into the pipette by the capillary action of the water. The isolated organism can then be removed and placed in a test tube of ceraphyl solution by gently blowing through the pipette. Here they will multiply to form pure cultures.

Caution the students to differentiate between the relatively faster moving protozoa and the usually slower moving multicellular animals such as rotifers.

ANSWERS TO QUESTIONS

1. Most plant cells contain green chloroplasts; animal cells do not. Most animal cells will be motile; most plant cells will not be. Plant cells will also have cell walls which are absent in animal cells.
2. Not in size or shape particularly; that is, multicellular organisms are not always larger than single-celled ones nor is there a special shape that is characteristic. The major difference is the subdivision of the body into many interdependent cellular units.
3. Students should not get the same types of organisms from various parts of the dish or various depths. For example, paramecia will be more common at the surface and amoebae at the bottom.
4. The student is to record the number of different types of one-celled organisms seen.
5. Movement will be by means of cilia, flagella, pseudopodia, or simply by floating in the currents—depending on the one-celled organism observed.
6. No. The means depend on the organism being observed.
7. Most organisms respond by a trial-and-error method of running into an object, backing away, changing course slightly and going forward again. This process is repeated until the object has been by-passed.
8. Cytoplasmic motion should be seen within the one-celled forms.
9. Few one-celled animals will have chloroplasts unless the teacher is using *Euglena*, *Chlamydomonas*, or similar forms which have a debatable taxonomic position.
10. When the water evaporates, the cell membrane of the organism ruptures and the animal dies.
11. They have cilia for locomotion. *Nyctotherus*, at least, will have an obvious nucleus and oral groove.
12. *Opalina* has no apparent point at which materials will enter the cell, *Nyctotherus* has a definite oral groove.
13. In *Opalina*, food is absorbed through the cell membrane.
14. They are adapted to living within the frog and have neither normal defensive mechanisms nor food-getting apparatus for effective free living.
15. Both are parasites. Neither one is adapted for a free-living existence. The structure differs, *Nyctotherus* having a definite oral groove and ingesting its food. *Opalina* has no specific point of food entry but ab-

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sorbs food through the cell membrane and therefore requires a nutrient medium for life. *Nyctotherus* might possibly do better as a free-living organism.

SUGGESTED READINGS

Books

Eddy, S. and A. C. Hodson. 1961. *Taxonomic Keys to the Common Animals of the North-Central States.* Burgess, Minneapolis.

Jahn, T. L. 1949. *How to Know the Protozoa.* W. C. Brown, Dubuque, Iowa.

Needham, J. and P. Needham, 1941. *Guide to the Study of Fresh-Water Biology.* Comstock, Ithaca, N.Y.

FILMS

Life in a Drop of Water, 1947, (sd, b&w or c, 11 min), p, Coronet

Life in a Cell, 1960, (sd, b&w or c, 14 min), p or r, Colburn

Protozoa: One-celled Animals, 1957, (sd, b&w or c, 11 min), r or p, EBF

Microbiology, Part II: *The Very Small*, No. 1, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Microbiology, Part II: *Complex Microorganisms*, No. 3, 1961, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

Microbiology, Part II: *Microorganisms with Chlorophyll*, No. 4, 1961, (sd, c, 32 min), p or r, AIBS(McGraw-Hill)

GENERALIZED CELL STRUCTURE

EXERCISE 3-8

TIME: One laboratory period.

MATERIALS: See page 29 in the *Student Laboratory Guide.*

■ **The purpose of this exercise is to help the student form his first generalization based on his laboratory observations of cells, and to help summarize the cell structures he has seen.**

SPECIAL INSTRUCTIONS

The usual approach to cell study in the laboratory is to present the student with a drawing of a generalized plant or animal cell *first* and then to ask him to examine cells in the laboratory in order to confirm or illustrate the textbook presentation. We hope that by proper scheduling the teacher can reverse this process, by first asking the students to look at various plant and animal cells to see the different structures in each cell type and *then* to summarize his observations in generalized drawings. After this, the student can go to the textbook for elaboration. Thus, we hope to inject an investigatory flavor into the laboratory work on cells rather than to follow the usual illustrative approach.

TECHNIQUES

Because the emphasis of this exercise is on the student's summarization of *his own* observations of cells, the teacher should forget the artistic quality of the drawings and should concentrate on the proper representation of the cell as the student has conceived it on the basis of observations. This exercise, since it requires no laboratory equipment, can be done as a culmination of the laboratory cell work and may conveniently be assigned as homework if so desired.

The students should be cautioned neither to copy pictures from their textbooks or other books nor to add objects they have not seen. You should keep in mind the limitations of the student's observations at this time and seriously criticize any drawings showing mitochondria, Golgi bodies, or an endoplasmic reticulum.

ANSWERS TO QUESTIONS

1. The student will probably list as structures in the generalized cell: nucleus, cytoplasm, vacuole, cell membrane, and cytoplasmic granules.
2. For the plant cell he should list chloroplasts and cell wall.
3. For the animal cell he may list such structures as contractile vacuoles, cilia, or an oral groove.
4. Plant cells have cell walls, which are lacking in the typical animal cell. Chloroplasts should not be included because they have not been in all plant cells examined (onion epidermis).
5. The animal cell most closely resembles the generalized cell because of its lack of chloroplasts and cell wall.
6. Further support for the hypothesis would be gained if observations of many different kinds of plants and animals showed that each one is composed of cells.
7. It would probably never be possible to prove the hypothesis simply because it would be impossible to examine every species of living plant and animal to be certain that they were made up of cells.

SUGGESTED READINGS

Books

Swanson, C. P. 1960. *The Cell.* Prentice-Hall, Englewood Cliffs, N.J.

FILMS

Cell Biology, Part I: *The Unit of Life*, No. 2, 1960, (sd, c, 30 min), p or r, AIBS (McGraw-Hill)

TYPES OF MICROSCOPES

EXERCISE 3-9

TIME: One laboratory period.

MATERIALS: See page 31 in the *Student Laboratory Guide.*

■ **The purpose of this exercise is to acquaint the student with the use of the stereoscopic dissecting microscope, to give an understanding of the various kinds of microscopes used in biological work, their limitations as to magnification and resolution, and a comprehension of some of the techniques employed in modern microscopy.**

SPECIAL INSTRUCTIONS

As the only manipulative procedures in this exercise involve the stereoscopic microscope, this exercise should be assigned as homework reading before coming to the laboratory. Here is placed together additional information dealing with the microscope in order to give the student a comprehensive view and review of the microscope and its use. The inclusion of large bodies of textual matter in the *Student Laboratory Guide* serves to emphasize to the student the unity of the textbook and laboratory work and breaks down the traditional use of the laboratory book for providing directions only for laboratory experiments.

TECHNIQUES

Earlier the student has been instructed in the use of the compound light microscope. Part A of this exercise instructs him in the use of the stereoscopic dissecting microscope. It acquaints him with the differences between the use of the stereoscopic dissecting microscope which does not reverse images and the compound one which does. The following suggested manipulations will develop some skill in the student in working with right and left hands under the stereoscopic microscope, as may be required later in this course:

Part A

Instruct the student as follows:

Sprinkle some salt from a shaker on a glass slide. Place the slide on the stage of the microscope so the salt is in view. Use a fine-pointed needle-like probe and arrange the salt crystals in the following ways: (a) in a straight line; (b) in a circle; (c) in a square.

Practice this with the right hand and then again with the left hand.

Place the following objects on a glass slide: Two tacks, straight pin, paper clip. Place the slide on the stage of the microscope and arrange the objects so you can see them.

Take a pair of forceps in the left hand and a pair in the right hand.

Do the following, being sure to use both hands as you practice:

a. Arrange the two tacks so the heads are together. Then reverse them so the sharp ends are together.
b. Pick up the clip with one of the forceps and the straight pin with the other. Run the straight pin through an opening in the clip.
c. Place the clip in the center of the field of vision. Pick up one tack with the forceps in the left hand and place the tack inside the loops of the clip so the sharp end is up. Do the same with the other tack but use the right hand.

ANSWERS TO QUESTIONS

1. and 2. The image seen through the stereoscopic microscope moves the same way as the object is moved, unlike the image seen through the compound microscope.
3. The image is larger under high power than low.
4. The size of the field is smaller under high power than low.
5. 12 to 4 sections.
6. 20,000 times total magnification.
7. No, the cell is too large to be seen at one time.
8. The combination of 10× ocular and 44× objective would give the magnification of 440×.
9. Chloroplasts.
10. Things appear larger but no extensive increase in detail can be noted within the cell parts.

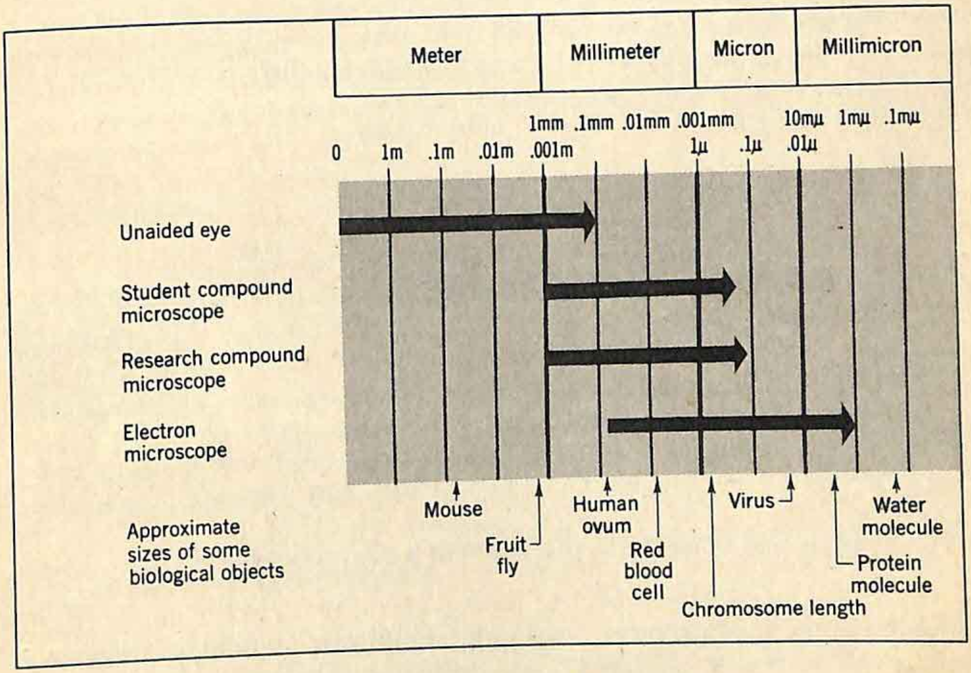


Figure 3-9-1 **Sizes of things.**

11. These would include the Golgi bodies, the mitochondria, and possibly crystals and vacuoles.
12. The grana vary from a few to several hundred and must be counted by the student.
13. At this magnification the grana appear simply as dark objects without visible internal structure.
14. At this magnification the grana can be seen to be dark bodies consisting of several layers.
15. This comparison from different aspects explains the difference in appearance of the same object.
16. B and C
17. E and F

SUGGESTED READINGS

Books

McClung, C. E. 1950. *McClung's Handbook of Microscopical Technique.* 3rd ed. Harper & Row, New York.

Articles

Brachet, J. 1961. "The Living Cell." *Sci. Am.* **205** [3] 51

Contains photographs taken with various kinds of microscopes.

FILMS

Microscope and Its Use, (sd, b&w, 10 min), YAF(McGraw-Hill)

Growth Cleavage (Maturation Division) in the Sperm Cells of the Grasshopper, Psophus stridulus *L.* (sd, b&w, 18 min), Brice

The Zernike film.

Phase Contrast Microscopy, (si, b&w, 14 min), B&L

Compound Microscope, 1953, (sd, b&w or c, 20 min), p, B&L

The Thinnest Slice, (sd, b&w, 21 min), p or r, USC

Histological Technique, 1952, (si, c, 30 min), Emory

SOME BASIC FUNCTIONS

EXERCISE

4-1

TIME: One period to set up, with subsequent observation time and a second period of testing for CO_2, measuring, and drawing conclusions.

MATERIALS: See page 41 in the *Student Laboratory Guide.*

Before using this exercise, particular emphasis should be placed on reading the textbook, Chapter 4 on Priestley, candles, mice, and mint, which this exercise largely parallels.

■ **The purpose of this exercise is to demonstrate the relationship of photosynthesis and respiration in an experiment which roughly parallels that of Priestley's experiment described in the textbook.**

SPECIAL INSTRUCTIONS

Because faulty techniques may cause this exercise to extend longer than the planned time, it should be started on a Monday so that it will not carry over a weekend.

One of the pitfalls in this exercise can be the effects of injury to the insects placed in the tubes. It is assumed that all of the insects are in equally good condition. If injury has occurred to any one of them, additional variables enter the picture and may negate the results. Of course, if this exercise can be replicated by a number of teams of students, the use of many insects will tend to cancel out these variables.

One way of handling the insects is to keep them in a bottle which is placed in the refrigerator shortly before the experiment is to begin. The cold will immobilize the insects and make them easier to handle.

Tubes used in this experiment should be illuminated day and night.

You should pre-test this exercise a few days in advance to determine approximate survival time of insects used, since local conditions introduce considerable variation.

The plug between the insect and the plant should not be so tight as to prevent the passage of oxygen generated by the plant into the chamber in which the insect is kept.

The students should be cautioned strongly about the NaOH pellets and provided with an appropriate tool for handling them. It will also be necessary to show them how to dispose of the NaOH solution and to see that the beakers and tubes have been thoroughly cleaned at the end of the experiment.

TECHNIQUES

This exercise may be performed as a demonstration if desired. If the tubes are small enough the insects should die within a relatively short time. It is important to conclude the experiment as soon after the death of the insect in Tube 1 as possible. Because the experiment may end after the class period, a succeeding class may conclude it and report back the results to the initiating class. A special student team or individual students can be assigned to stop by the biology room during the day to observe how long the insects in Tubes 1 and 3 live.

ANSWERS TO QUESTIONS

1. Tube 4 functions as a control.
2. The insect in Tube 3 should live longer because the plant provides a source of oxygen denied the insect in Tube 1.

3. From student data.
4. From student data.
5. By testing with a glowing splint for oxygen or bubbling the gas through limewater to test for CO_2. The NaOH test which follows is a test for CO_2.
6. The water level should rise in the tube as carbon dioxide is absorbed by the NaOH.
7. The insect used up the available O_2 and then died. As the insect used up the O_2, it also gave off CO_2, which replaced the volume of O_2 used. After the NaOH was added to absorb the CO_2, the volume of CO_2 was measured in millimeters by the rise of water in the tube.
8. In Tube 2 the leafy plant shoot should generate enough oxygen in the light to cause an increase in the volume of air in the tube. The amount of carbon dioxide should be less than in Tube 1, since there was no insect to produce it and the plant is using more CO_2 than it produces. It should be possible to demonstrate this by the NaOH test.
9. The insect in Tube 3 should live the longest because of a net balance between oxygen produced by the plant and used by the insect, and carbon dioxide produced by the insect and used by the plant. The volume change will vary with the amount of photosynthesis and the activity of the insect if all other factors are constant. Carbon dioxide should be noticed in the NaOH test, but it should not be as abundant as it was in either Tubes 1 or 2.
10. In Tube 4 there should be no appreciable changes except those occasioned by temperature. Carbon dioxide should be present in the quantity normally present in the air.
11. Changes in air temperature will influence the volume of gas in Tube 4, and there is a possibility that the sugar in the filter paper may ferment, altering the volume of gas in the tube by releasing CO_2, if microorganisms are present.
12. The data obtained by measuring Tubes 1, 2, and 3 would have to be corrected by subtracting the amount of change seen in Tube 4.
13. Basic observations should include the fact that the plant produces oxygen, which is needed by the insect in respiration. The insect produces carbon dioxide needed by the plant in photosynthesis. While the plant can live without the insect, the insect cannot live without the plant.

SUGGESTED READINGS

Books

Schwartz, G. and P. W. Bishop. 1958. *Moments of Discovery, Vol. I, Origins of Science.* Basic Books, New York
Contains the work of Joseph Priestley and Antoine Lavoisier.

Articles

Gray, G. W. 1955. "Life at High Altitudes." *Sci. Am.* **193** [6] 58
Williams, C. M. 1953. "Insect Breathing." *Sci. Am.* **188** [2] 28

FILMS

Cell Biology, Part I: *Cell Respiration*, No. 5, 1960, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

The Multicellular Animal, Part IV: *Respiration*, No. 4, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Role of the Green Plant*, No. 5, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)

THE ANALYSIS OF WATER

EXERCISE 5-1

TIME: One laboratory period.

MATERIALS: See page 43 in the *Student Laboratory Guide.*

This exercise is designed to accompany Chapter 5 of the textbook, particularly the sections on the analysis of water and the section dealing with energy in water.

■ **The purpose of this exercise is to demonstrate an analysis of water similar to the splitting of water in the process of photosynthesis and to show the chemical composition of water as a compound. The demonstration will reveal some of the properties of hydrogen and oxygen and the energy relationship between chemical analysis and synthesis.**

SPECIAL INSTRUCTIONS

This exercise is intended as a demonstration to be performed by the teacher. However, it is described fully in the *Student Laboratory Guide* in case you wish to assign it to a student or a group of students to perform as a class demonstration or individual exercise.

Special care must be taken in handling the concentrated sulfuric acid, which is highly corrosive.

Special care must be taken to insure that the hydrogen and oxygen gases released by the apparatus are not mixed. Mixing the two will result in a violent explosion.

When a lighted splint is held near the mouth of the test tube containing hydrogen, a mild explosion occurs. Do not hold your hand near the mouth of the tube. Hold the splint by its far end.

The students may have seen this demonstration previously in a general

SCHEDULE OF EXERCISES FOR GENETICS AND EVOLUTION

Week	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
1					30-3 Original mating
2	30-1		30-2		30-3 Remove parents
3		30-3 Examine and mate F_1			30-4 Original mating
4	31-1 Original mating	30-3 Remove F_1 parents		31-2 Original mating	30-4 Remove parents 30-3 Begin to examine F_2
5	31-1 Remove parents	30-4 Examine and mate F_1	Begin 30-5	31-2 Remove parents	31-1 Examine and mate F_1
6	31-2 Examine and mate F_1	30-4 Remove F_1 parents		Start observations for 30-5	30-4 Examine F_2 31-1 Remove parents
7	31-2 Remove parents	31-1 Examine F_2	Finish 30-5		31-2 Examine F_2
8	Assign 32-2		Assign 33-1	Begin 32-1	Finish 32-1
9	Assign 33-2		Assign 34-1		Assign 34-2
10	Assign 36-1		Do 36-2		

DROSOPHILA TECHNIQUE

EXERCISE
30-1

TIME: One laboratory period.

MATERIALS: See page 213 in the *Student Laboratory Guide.*

It is essential to perform this exercise before any of the other *Drosophila* exercises, which are designed to accompany Chapter 30 of the textbook.

■ **The purpose of this exercise is to familiarize students with the development and metamorphosis of an insect, as well as to acquaint them with the techniques of handling and culturing *Drosophila* and to learn to distinguish the normal wild-type males and females.**

SPECIAL INSTRUCTIONS

This exercise is a necessary introduction to genetics laboratories if *Drosophila* is to be used. Unless the student masters the techniques of handling, culturing, and recognizing male and female *Drosophila*, work in future exercises will be seriously handicapped.

In order to avoid confusion, it is desirable to have developing cultures (vials) of about 50 living fruit flies of the wild variety for each student or student team. These will have to be prepared in advance of the exercise.

Fresh vials in which the flies have been introduced a few hours previously are advantageous for showing the eggs. Older cultures with larvae, pupae, and adults should also be available.

For short periods of time, such as a school day, the flies may be put into dry vials.

TECHNIQUES

Equipment Needed for Culturing *Drosophila*

The following equipment should be collected before preparing the medium.

Culture bottles: Half-pint bottles or 250-ml wide-mouthed collecting bottles can be used for keeping stocks, and shell vials (about 80 mm x 23 mm) for individual crosses. Two dozen bottles should be enough for stock maintenance, plus perhaps a gross of vials for each cross undertaken (for five or six sections)

Nonabsorbent cotton, 2 or 3 pounds, cheapest grade

Cheesecloth, cheapest grade, for enclosing the cotton plugs

A 1000 ml (or larger) Pyrex flask with a wicker covering. The wicker covering allows you to pour from the flask while the medium is hot

Iron tripod support stand, or ring stand

Bunsen burner (Meeker burner is much better) science course. If so, it can be repeated with emphasis on the relationship between the electrolysis demonstrated here, and the photolysis of water in the photosynthetic reaction.

The Hoffman electrolysis apparatus is inexpensive and can be purchased complete with platinum electrodes. However, as it is a common item in the chemistry laboratory, it may be borrowed for this exercise or the chemistry teacher may be induced to demonstrate it.

TECHNIQUES

Dry-cell batteries can be used as a direct current source of approximately 6 to 10 volts or a direct current rectifier can serve as a source of current.

The indication of water levels before and after the experiment is essential for comparative purposes.

ANSWERS TO QUESTIONS

1. After the addition of sulfuric acid, bubbles form at the platinum electrodes and gas is evolved.
2. The amounts in the tubes are not the same.
3. One has twice the volume of the other.
4. Yes.
5. Electrical energy.
6. More, for they are capable of combining again with a subsequent release of energy.
7. An explosion takes place.
8. The presence of hydrogen, for which a test was indicated in the introduction to the exercise.
9. Droplets of water.
10. Hydrogen has combined with the oxygen of the air to form water.
11. An explosion is a release of energy.
12. Synthesis.
13. It burns brightly.
14. Oxygen supports combustion.
15. Yes.
16. Heat.
17. The one with the greater gas volume.
18. The one with the lesser gas volume.
19. The volume of hydrogen bound in the water molecule is greater than the volume of bound oxygen.
20. Both reactions break water into hydrogen and oxygen. One uses light energy, the other electrical energy. One requires chlorophyll, the other platinum electrodes and sulfuric acid. In electrolysis both gases are freed; in photolysis only oxygen is freed.
21. No.
22. The hydrogen combines with the carbon dioxide to form a carbohydrate—in this case, glucose—as well as water.

SUGGESTED READINGS

Books

Asimov, I. 1958. *Inside the Atom.* Abelard-Schuman, New York

Articles

Barker, E. F. 1946. "Energy Transformations and the Conservation of Mass." *Am. J. Phys.* **14** p. 309.

DeVault, D. 1944. "A Method of Teaching the Electronic Structure of the Atom: Advanced Topics." *J. Chem. Ed.* **21** p. 575

Scott, J. H. 1959. "The Nineteenth Century Atom: Undivided or Indivisible." *J. Chem. Ed.* **36** p. 64

FILMS

Chemistry of Water, 1960, (sd, c, 14 min), r, Indiana
Molecular Theory of Matter, 1932, (sd, b&w, 11 min), p or r, EBF
Properties of Water, 1947, (sd, b&w or c, 11 min), p, Coronet
Hydrogen, 1959, (sd, b&w or c, 13½ min), p, Coronet
Oxygen, 1947, (sd, b&w or c, 11 min), p, Coronet

DIFFUSION THROUGH A MEMBRANE

EXERCISE 6-1

TIME: One laboratory period.

MATERIALS: See page 45 in the *Student Laboratory Guide.*

This exercise is designed to accompany the sections dealing with enzymes and with the cell membrane and diffusion in Chapter 6 of the textbook.

■ **The purpose of this exercise is to familiarize the student with the concept of a semipermeable membrane and with the process of diffusion. In addition, enzyme activity is introduced and demonstrated by a diffusion technique.**

SPECIAL INSTRUCTIONS

The dialysis tubing and its contents are to be considered a crude model of a cell. While the student portion of the exercise does not compare the model to a cell, nonetheless, teachers should attempt, in introducing the exercise, to clarify the problem of using models to simulate living systems and functions. Whatever you do, do not give this exercise to students without relating it to living organisms.

For the glucose tests the amount of water outside the cellophane bag should be as small as possible, otherwise the sugar diffusing out into it may be too dilute to yield a clear test. In Part B the Benedict's solution must be used, because Clinitest tablets or Tes-Tape will not give the appropriate reaction.

Both glucose and iodine are used so that the students may understand that materials can diffuse simultaneously in opposite directions through a semipermeable membrane.

To save both time and materials, students can be assigned to work in teams of any desired size. The materials listed in the *Student Laboratory Guide* are per team.

TECHNIQUES

In order for the students to know what to expect from sugar and starch tests, these tests should be performed as a demonstration before the student is asked to do them.

The entire exercise may be done as a demonstration. In that case Part C should be set up before the first class of the day in order to let sufficient time elapse to show a rise in the column in the pipette.

Be sure the bags are tied securely; otherwise leakage will occur and the effect of the exercise will be destroyed.

Before class time soak the 5/8-inch dialysis tubing thoroughly in water and cut 20-cm lengths after soaking. Do not attempt to unroll, flatten, or otherwise straighten the dry tubing, for it may crack and the leaks will allow free passage of all materials in and out of the bags.

The diagram in the laboratory exercise shows the set-up for the equipment. Bottles are used as the containers for immersing the dialysis tubing. If large test tubes are used instead of bottles, a rack for the tubes must be provided for each team of students.

Prepare approximately 1 liter of 80% glucose solution. This quantity should be sufficient for approximately 75 teams of two students each. The quantity can be adjusted as necessary.

The soluble starch solution is prepared by adding 80 g of soluble starch to about 500 ml of water. Stir or shake, then filter to get a clear solution. Boiling the starch solution insures a thorough mixture. If you cannot obtain soluble starch, substitute laundry starch and filter it through a cloth fabric and finally through filter paper.

In the exercise involving the test for sugar, pipette glucose from the bottom of the container where it is more concentrated.

Note that the term *osmosis* is not used either in the laboratory or in the text. The term *diffusion* sufficiently covers the process commonly designated as osmosis and eliminates an unnecessary term.

As a substitute for the dialysis tubing, collodion can be formed into a bag by pouring it into a test tube and removing it with cold water. However, commercial dialysis tubing is so inexpensive and so much easier to use that the collodion technique is not recommended.

When the equipment and materials are set up correctly, iodine will diffuse into the contents of the cellophane tubing and a dark color will become apparent in the starch. Some glucose will diffuse from the dialysis tubing and can be detected in the surrounding water.

The enzymes in saliva will act on the starch, and the resulting sugar (maltose) will diffuse through the membrane into the external solution, where its presence can be detected by Benedict's solution. If you or your students prefer, a solution of diastase can be substituted for the saliva with satisfactory results and less trauma to delicate psyches.

ANSWERS TO QUESTIONS

1. Tube should swell slightly as water passes into it, but the most dramatic observation will be the color change as iodine diffuses into the cellophane bag and reacts with the starch.
2. The iodine diffused into the cellophane bag.
3. The starch remained in the cellophane bag and did not diffuse out because no color change indicative of starch appears in the water.
4. The glucose diffused out of the bag.
5. Iodine, glucose, water.
6. Starch.
7. The membrane has pores of a certain size. The molecules of starch were too large to pass through the pores of the membrane.
8. That the membrane must be perforated by openings smaller than starch molecules.
9. Yes, and at different rates and amounts.
10. The iodine diffused in; the glucose diffused out.
11. As Part A showed that starch did not diffuse out of the tube and as no glucose was added to this tube, one must conclude that the saliva acted on the starch to change it to sugar (maltose) which diffused through the membrane, whereupon its presence was detected by testing the water with the Benedict's solution.
12. The water diffuses into the cellophane tubing much more rapidly than glucose diffuses out. The difference causes the level of the solution in the tubing to rise. The data plotted on a simple graph show this dramatically.

SUGGESTED READINGS

Articles

Holter, H. 1961. "How Things Get into Cells." *Sci. Am.* **205** [3] 167

Ingle, R. M. 1954. "The Life of an Estuary." *Sci. Am.* **190** [5] 64

Siekevitz, P. 1957. "The Powerhouse of the Cell." *Sci. Am.* **197** [1] 131

FILMS

Cell Biology, Part I: *Transfer of Materials*, No. 7, 1960, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

Osmosis, 1958, (sd, b&w or c, 14 min), p or r, EBF

ACTIVITIES OF THE CELL MEMBRANE

EXERCISE 6-2

TIME: One laboratory period.

MATERIALS: See page 49 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on the activities of the cell membrane and diffusion in Chapter 6 of the textbook.

■ **The purpose of the exercise is to demonstrate diffusion and the semipermeability of the living cell membrane in actual cells, in contrast to the use of a model in Exercise 6-1.**

SPECIAL INSTRUCTIONS

Exercise 6-1 involved a study of a semipermeable membrane and diffusion in a cell model. This exercise involves semipermeable membranes and diffusion in actual cells, living and dead.

Dead cells such as those produced by boiling, lose the selective permeability of their living cell membrane, and the dye (Congo red) readily passes into the cell. The membranes of living cells, however, prevent the entrance of Congo red.

TECHNIQUES

The proper scientific name of the plant used is *Anacharis.* It is commonly referred to as elodea.

To prepare a yeast suspension, add 1 g of peptone and a half a cake of dry yeast to 500 ml of a 5% to 10% solution of molasses or diluted grape juice. Culture for at least 12 hours prior to class use at a temperature of 25° to 30° C.

Caution the student not to place too much salt solution on the slide for Part B, for spilled solutions will have a deleterious effect on the microscope.

In Part B the diffusion of the water from inside the cell will cause the cytoplasm to shrink away from the cell wall. This is termed *plasmolysis*, but we advise against the use of this unnecessary term. When the leaf is returned to plain water, the cell will again fill with water and will be restored to its normal condition. If the leaf is allowed to remain in salt water for 15 minutes or longer, the cellular material will remain in a condition of plasmolysis and die.

ANSWERS TO QUESTIONS

1. The cells which were boiled.
2. There is a red color in the dead cells, caused by the entrance of the Congo red dye.
3. The membrane of a living cell does not allow Congo red to enter.
4. The cytoplasm shrinks away from the cell wall.
5. The cell returns to its normal condition.
6. The cell dies and the cytoplasm remains in a contracted state.
7. All streaming of the cytoplasm has stopped.
8. That a living cell membrane is semipermeable, that is, it allows the entrance of certain substances but not others (Congo red). That it allows the free passage of water in and out and is apparently not selectively permeable toward water.
9. This is probably due to molecular size. The water molecules are small enough to pass easily in either direction, but the Congo red molecules can be kept out. The selectivity of the living membranes versus the nonselectivity of the dead one must be taken into account.
10. The water molecules are of small size. Congo red molecules are normally too large to pass through the living membrane. Refer again to selectivity of the membrane.

SUGGESTED READINGS

Books

Ferry, J. F. and H. W. Ward. 1959. *Fundamentals of Plant Physiology*. Macmillan, New York

Meyer, B. S., D. B. Anderson, and R. H. Boning. 1960. *Plant Physiology*. Van Nostrand, New York

Swanson, C. P. 1960. *The Cell*. Prentice-Hall, Englewood Cliffs, N.J.

Articles

Robertson, J. D. 1962. "The Membrane of the Living Cell." *Sci. Am.* **206** [4] 64

FILMS

Cell Biology, Part I: *Transfer of Materials*, No. 7, 1960, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

Effects of Metallic Ions and Osmotic Disturbances on the Heart, (sd, b&w, 12 min), r, Indiana

Osmosis, 1958, (sd, b&w or c, 14 min), p or r, EBF

SOME SPECIFIC COMPOUNDS IN CELLS

EXERCISE 6-3

TIME: One laboratory period.

MATERIALS: See page 51 in the *Student Laboratory Guide.*

This exercise is to accompany the section dealing with glucose in Chapter 6 of the textbook.

■ **The purpose of the exercise is to detect the presence of sugar and starch in plant cells and to determine the location of these compounds within the plant.**

SPECIAL INSTRUCTIONS

It is extremely important that the students prepare thin sections; otherwise their microscopic observations will be useless.

The student should be familiar with the tests for starch and sugar, from Exercise 6-1.

Almost any color change with Benedict's solution will indicate the presence of sugar—the longer the time the more the indicator will approach the brick-red condition.

Care should be taken that the student does not use the same knife for cutting both the apple and the potato, since there may be some transfer of materials from the surface of one to the surface of the other by means of the knife.

TECHNIQUES

Hard apple butter may be substituted for the apple itself, if desired.

A candle can be used in lieu of an alcohol lamp or Bunsen burner, but care must be taken to see that soot does not accumulate on the bottom of the slide.

There is frequently a tendency on the part of students to heat the slide too much and thus cook the preparation. It is desirable to test the temperature of the slide by removing it from the flame at intervals and placing it on the back of the hand. When it feels warm to the touch this is sufficient heating.

ANSWERS TO QUESTIONS

1. The iodine turns bluish brown to bluish black in the presence of starch.

2. Not all of the bodies contain starch. Those that do contain starch do not contain it in the same amount.
3. No, it is simply a test for the presence of starch and nothing else. Many other substances can be found.
4. There has been a color change from green to brick-red.
5. No, apparently it is present in all cells.
6. The starch is in special cell structures (plastids) in the potato, but sugar is not confined to specific cell structures in the apple.
7. Yes, there is sugar in the potato.
8. Yes, there is starch in the apple.

SUGGESTED READINGS

Books

Downes, H. R. 1962. *The Chemistry of Living Cells.* Harper & Row, New York
Chapter 15 deals with carbohydrate metabolism.

Articles

Pfeiffer, J. E. 1948. "Enzymes." *Sci. Am.* **179** [6] 28

FILMS

Cell Biology, Part I: *From Atoms to Organisms*, No. 12, 1960, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)
A small portion of this film deals with carbohydrates and the building of structure.

Multicellular Plants, Part III: *Role of the Green Plant*, No. 5, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)
Deals in part with carbohydrates and plant metabolism.

Cell Biology, Part I: *Patterns of Energy Transfer*, No. 6, 1960, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)
A part of the film discusses carbohydrates.

ENZYMES IN LIVING TISSUE

EXERCISE 6-4

TIME: One laboratory period.

MATERIALS: See page 53 in the *Student Laboratory Guide.*

This exercise is to accompany the section that discusses enzymes in Chapter 6 of the textbook.

■ **The purpose of the exercise is to demonstrate the presence of the enzyme catalase in different living materials and its absence in non-**

living materials. The demonstration in Part B will show the presence of a starch-digesting enzyme (amylase) in living plant tissue.

SPECIAL INSTRUCTIONS

Preparation for Part B must start three days prior to the scheduled laboratory.

To save time and materials, students may be assigned to work in groups of a desired size.

For Part A the hydrogen peroxide solution must be fresh. It should be stored in dark bottles and kept out of sunlight.

In order to accomplish both the student part of the exercise (Part A) and the demonstration (Part B), materials should be available for the students at the beginning of the period. They should be told to read Part A before coming to laboratory. The demonstration materials must be prepared prior to class time.

The materials in this exercise will correlate well with future exercises on enzymes.

TECHNIQUES

This exercise can be accomplished by placing a tiny piece of tissue to be tested on a glass slide, in lieu of test tubes, and by adding to it one drop of hydrogen peroxide. The reaction can still be seen, and test tubes, tissue, and H_2O_2 are saved.

If the students filter ground tissues mixed in water, you should first demonstrate the process of folding filter paper and filtering. Paper toweling can be substituted for filter paper.

The formula for the reaction of hydrogen peroxide with the enzyme catalase is as follows:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

The oxygen atoms immediately combine to form oxygen molecules and these bubble off, causing the foaming.

Liver is an especially good tissue with which to demonstrate the catalase reaction.

For the demonstration, start soaking corn grains at least three days before the demonstration. Have enough for every member of the class plus some for use on the Petri dishes. While the grains are soaking, prepare starch agar and pour a fairly thin layer in each of three or more Petri dishes. Prepare one or two dishes of plain agar to be used as a control.

Take two germinating corn grains. Cut them longitudinally and parallel to the flat surfaces and place them with their cut faces on the agar. At least two days should elapse from this time to the time they are used in class.

Take some of the germinating seeds and kill them in FAA solution by soaking for 24 hours. Cut two of these grains in the same way as the germinating grains were cut and put them on starch agar plates as controls.

As this is the first time the students have heard the terms *Petri dish* and *agar* you may wish to explain them, although they are covered later in detail in the microbiology section of the laboratory.

Make sure the students understand that the iodine solution turns blue in the presence of starch.

ANSWERS TO QUESTIONS

1. Bubbles form (of oxygen).
2. Yes.
3. No.
4. Depends on the student's materials. By grinding the tissue the student will see that the enzyme works even when it is not in a cell. He will see that it will be inactivated by acid, alkali, or heat because the killing agents have destroyed the protein enzymes, and no enzymatic action remains possible.
5. There is no bubbling reaction with inert materials.
6. Catalase occurs in all the living materials tested but not in the nonliving materials. The enzyme can be inactivated by severe environmental changes such as extremes of pH or high temperature.
7. Control.
8. Data.
9. A hypothesis.
10. The iodine test shows an abundance of starch present in both living and dead corn grains.
11. Protein, sugar, and corn oil are not demonstrated by this test.
12. The starch agar shows a positive test (blue color) when flooded with iodine. There is no color change (no starch) in the plain agar dish.
13. An enzyme (amylase) formed in the tissue of the germinating corn grain has diffused from the cut surface of the grain into the agar and has digested the starch. Because there is no starch present in this part of the agar, it remains clear after the iodine test for starch is applied.
14. The result of starch digestion, a sugar.
15. No clear areas because the starch digesting enzyme was inactivated chemically so the starch was not broken down.
16. No, because moisture is necessary as a medium for enzyme activity.

SUGGESTED READINGS

Books

Downes, H. R. 1962. *The Chemistry of Living Cells.* Harper & Row, New York

Articles

Bearn, A. G. 1956. "The Chemistry of Hereditary Disease." *Sci. Am.* **195** [6] 126
Gale, E. F. 1956. "Experiments in Protein Synthesis." *Sci. Am.* **194** [3] 42
Green, D. E. 1949. "Enzymes in Teams." *Sci. Am.* **181** [3] 48
—— 1954. "The Metabolism of Fats." *Sci. Am.* **190** [1] 32
Meyerhof, O. 1950. "Biochemistry." *Sci. Am.* **183** [3] 62
Pfeiffer, J. E. 1948. "Enzymes." *Sci. Am.* **179** [6] 28

FILMS

The Multicellular Animal, Part IV: *Ingestion and Digestion*, No. 3, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Only a small part of this film deals with digestive enzymes.

ENZYME ACTION ON A PROTEIN

EXERCISE 6-5

TIME: Part of two laboratory periods.

MATERIALS: See page 55 in the *Student Laboratory Guide.*

This exercise is to accompany the section that discusses enzymes in Chapter 6 of the textbook.

■ **The purpose of the exercise is to demonstrate the action of enzymes on proteins, to indicate some of the factors limiting enzyme action, and to develop an understanding of and some competence in the techniques of paper chromatography.**

SPECIAL INSTRUCTIONS

It is necessary to prepare test mixtures I and II 48 hours before the testing is to be performed. The students can then do the work as outlined. As it takes approximately an hour for the solvent to rise sufficiently, the strips may have to be removed from the test tubes during the following period.

A second period for developing the chromatogram and drawing conclusions is required.

The demonstration with gelatin and pineapple juice can be performed while waiting for the solvent to rise or the paper strips to dry.

In performing the paper chromatography it is advantageous to use the BSCS film on chromatographic techniques to demonstrate the procedures involved.

The paper used for chromatography should always be handled with forceps in order to avoid fingerprints which will give a positive test for amino acids.

When heating the strip during developing, care must be taken not to burn the paper.

The fumes of the ninhydrin solution are toxic. Care should be taken that this part of the exercise is performed near an open window in a well-ventilated room, or under a hood if available. To avoid possible difficulties the teacher should perform this developing technique.

TECHNIQUES

This exercise works well with the students divided in groups or pairs.

The slit technique of fastening the paper to the cork is the preferable one, although the paper may be tacked or taped to the cork. To prevent curling, the paper strips should be placed in an envelope and pressed beneath a book until ready to use. A tack or paper clip at the bottom of the strip will prevent it from curling in the tube, but care must be taken that rust from the tack or clip does not interfere with the chromatogram.

The ninhydrin reagent can be sprayed on the paper instead of immersing the strip in it.

Part B is best done as a demonstration while the students are waiting for portions of Part A to proceed.

Jello or other commercially available gelatin dessert will serve as gelatin for this demonstration.

A variety of solvents can be substituted for the one called for under Materials in the *Student Laboratory Guide*. Absolute ethyl alcohol and water (3:1) or water-saturated phenol are suggested substitutes.

Chromatography paper can be purchased in strip rolls or in sheets from a biology supply house, but strips can be cut from ordinary filter paper because separation of the individual amino acids is not important in this exercise.

If students work in pairs or groups, each student or group should prepare one of the two chromatograms.

In student discussions be sure to emphasize the specificity of enzymes, the effect of heat on enzymes, and the fact that chromatography is an important analytical technique in biology.

ANSWERS TO QUESTIONS

1. Yes. Spots of purple color do appear.
2. No purple spots appear here.
3. Boiling the juice in Test II destroys the enzyme; as a consequence no protein hydrolysis takes place to free amino acids from the protein.
4. An enzyme from the pineapple might prevent gelling by digesting gelatin, if it is a protein, to form amino acids which do not gel.
5. A protein.
6. As seen in this experiment, the cooking inactivates the enzyme. Canned pineapple juice is cooked when processed.
7. The gelatin to which the fresh pineapple has been added remains soft and watery; the other has begun to gel.
8. The enzymes in the fresh frozen pineapple juice digest the gelatin to amino acids, thus preventing gelling. The cooking inactivates the enzyme in the second case.

SUGGESTED READINGS

Books

Boyer, P. D., H. Lardy, and K. Mybäck. 1959–63. *The Enzymes* (7 vols.) Academic Press, New York

Colowick, S. and N. Kaplan. 1955–62. *Methods in Enzymology* (5 vols.) Academic Press, New York

Downes, H. R. 1962. *The Chemistry of Living Cells.* Harper & Row, New York
Chapter 7 deals with enzymes.

Hardin, G. 1961. *Biology: Its Principles and Implications.* W. H. Freeman, San Francisco
Pages 61 through 65 deal with energy and enzymes.

Articles

Pfeiffer, J. E. 1948. "Enzymes." *Sci. Am.* **179** [6] 28

FILMS

Paper Chromatography, 1961, (sd, c, 14 min), p or r, (BSCS) Thorne

FACTORS INFLUENCING ENZYME ACTION

EXERCISE 6-6

TIME: One to four laboratory periods.

MATERIALS: See page 59 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on enzymes in Chapter 6 of the textbook.

■ **The purpose of this exercise is to demonstrate the influence of temperature, dilution, pH, and time on the action of an enzyme (amylase).**

SPECIAL INSTRUCTIONS

One or two periods will be needed, depending upon the availability of equipment and how the class is divided into teams. If all members of the class do each of the four parts, this exercise takes four periods.

Students can be referred back to Exercise 3-2 concerning the metric system, or to Appendix II in the *Student Laboratory Guide* which deals with the conversion of Fahrenheit to Celsius units, if they are not familiar with the Celsius system. *Celsius* is preferred to the term *Centigrade*, with which it is identical.

Saliva is used as an enzyme source in these exercises to show the existence of an enzyme in a secretion produced by the human body. In the beginning, the students may react with distaste to contributing saliva but by the time the exercise has progressed a bit, it becomes a commonplace laboratory procedure. It is possible to substitute diastase (0.2% in water) or pancreatin (1%) as a substitute for saliva.

The useability of this exercise depends primarily on good class organization. If sufficient equipment is available and the class is large enough to divide into four groups, the following equipment can be distributed to each group and each group asked to perform one of the four parts, with a subsequent discussion of the results with the whole class.

Check List of Materials and Equipment for Parts A, B, C, and D
(+ means required; — means not required; numbers indicate quantity)

	Part A	Part B	Part C	Part D
	Temp.	*Time*	*Dilution*	*pH*
Test tubes	6	4	6	16
Test-tube holder	2	2	2	2
Test-tube rack	1	1	1	2
Funnel	1	—	—	—
Filter paper	Box	—	—	—
Iodine solution in dropper bottle	1	1	1	1
Benedict's solution in dropper bottle	1	1	1	1
Crackers (sugar-free)	4	—	—	—
Starch solution	—	10 ml	6 ml	32 ml
Collect saliva as needed	+	10 ml	1 ml	16 ml
Paraffin	+	+	+	+
Ice	+	—	—	—
Water bath complete:	—	—	1	—
Thermometer	2	—	1	1
One 250-ml beaker	—	1	1	—
One 500-ml beaker	3	—	1	1
Bunsen burner	3	2	2	2
Tripod stand and screen	3	1	1	1
One 100-ml beaker	1	1	—	—
10-ml graduated cylinder	—	1	1	—
Slides or watch glasses	1	1	1	1
1-ml pipette	—	—	1	2
Droppers	4	4	4	4
0.1 *M* HCl	—	—	—	10 ml
0.1 *M* NaOH	—	—	—	10 ml
Wax pencil	—	—	1	1

The presence of chewing gum or food in the mouth will give a positive sugar test with saliva. Students should be asked to rinse their mouths before contributing saliva.

TECHNIQUES

Check the ingredients listed on the box of soda crackers used for this experiment. If sugar is present, the crackers are not suitable.

Fehling's solution may be substituted for Benedict's in this experiment.

In order to standardize the results, place on the blackboard the format you wish used for the tabular recording of data (step 9 Part C; step 7 Part D).

Substitutions for glassware can be made wherever feasible. Slides can substitute for watch glasses, watch glasses in turn for test tubes, depending upon the amount of glassware available.

ANSWERS TO QUESTIONS

1. Amylase action has changed starch into something else.
2. Sugar.
3. The starch solution does not pass through the filter paper but the sugar does.
4. Starch grains are too large to pass through the filter paper; the glucose molecules are not.
5. The test is negative in the boiled tube, weak in the tube heated to 60° C, and positive in the tube heated to 37° C.
6. The enzyme amylase has been inactivated by boiling.
7. The enzyme has an optimum temperature for activity. It is much more effective at body temperature than at either extreme.
8. Enzymes react rapidly.
9. Depends on student's observation, but very small amounts of enzyme are effective.
10. The enzyme will not react well in strong acid or strong base solutions. It possesses an optimal pH nearer the neutral point.
11. Temperature, time, dilution, and pH all affect the action of enzymes. Temperature extremes and extremes of pH tend to inhibit enzyme action or stop it entirely. While enzymes can act in extremely dilute amounts, nonetheless the action is not as pronounced as when more enzyme is present. Enzymes act rapidly and with the passage of time will catalyze all of the substrate and thus will produce no further reaction if the experiment is carried on too long.

SUGGESTED READINGS

Books

Lawson, C. A. *et al.* 1957. *Laboratory and Field Studies in Biology.* National Research Council, National Academy of Sciences, 2101 Constitution Ave., N.W., Washington, D.C., p. 343

Morholt, E., P. Brandwein, and A. Joseph. 1958. *A Sourcebook for the Biological Sciences.* Ch. 4. Harcourt, Brace & World, New York

Simpson, G. G., C. Pittendrigh, and L. E. Tiffany. 1957. *Life: An Introduction to Biology.* Ch. 6. Harcourt, Brace & World, New York

Articles

Adler, L. K. 1959. "Introduction to Enzyme Activities." *Science Teacher.* October, p. 431

FILMS

Catalysis, 1937, (sd, b&w, 10 min), p or r, EBF

Cell Biology, Part I: *Cell Respiration*, No. 5, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Cell Biology, Part I: *Growth and Replacement*, No. 8, (sd, c, 30 min), p or r, AIBS (McGraw-Hill)

Chemistry Series, Modern: *The pH Scale*, Lesson 57, 1959, (sd, b&w or c, 30 min), p or r, EBF

Chemistry Series, Modern: *Proteins–Enzymes*, Lesson 81, 1959, (sd, b&w or c, 30 min), p or r, EBF

A part of each of these films is concerned with enzymes.

MITOSIS IN PLANT AND ANIMAL CELLS

EXERCISE 7-1

TIME: Two laboratory periods.

MATERIALS: See page 63 in the *Student Laboratory Guide*.

This exercise is to be done in conjunction with the work on plant and animal mitosis in Chapter 7 of the textbook.

■ **The purpose of this exercise is to demonstrate the quantitative and qualitative nature of nuclear division which occurs during the process of mitosis in both plant and animal cells. It is aimed at understanding the nuclear events which determine that the two daughter cells will be identical in hereditary nature.**

SPECIAL INSTRUCTIONS

Divide the class into working groups commensurate with the time and materials available.

In teaching students the nature of mitosis, avoid emphasis on non-essential details and on learning the names of phases such as prophase, metaphase, anaphase, telophase, and interphase. These are convenient terms, *after* one has learned that the essential features of mitosis are the replication of chromosomes, followed by the separation of the twin or daughter chromosomes to the opposite poles of a spindle. Emphasize the identity of chromosomal make-up between the two daughter nuclei that result, and the identity of each of them with the parent nucleus. Point out that the cytoplasm is not necessarily divided equally. Sometimes it is; sometimes it is not. This brings out the identity of the genetic material in the parent cell and all cells that descend from it. Emphasize cellular continuity through mitosis as the basis of genetic continuity (heredity), as the basis of development, and as the basis of reproduction. Asexual reproduction, by fission or budding, results in progeny identical to each other and to the parent because mitosis is the only process of nuclear reproduction involved. Thus the process of mitosis insures genetic identity of the descendant cells.

By all means use a movie of mitosis to give students an idea of the active, exciting "dance of the chromosomes" on the spindle as they become attached to it and then part company.

TECHNIQUES

Part A: Smears of Developing Tradescantia *Pollen*

Tradescantia flowers, either fresh or preserved in Carnoy's solution, can be used for this exercise. We encourage all teachers to obtain and cultivate in the laboratory this common plant, which has many uses as an experimental organism.

Tradescantia pollen is used here because its nucleus divides when the flower buds are about 5 mm long, and there are six beautiful, large chromosomes. The double nature of these chromosomes can easily be seen in metaphase. The only difficulty to be encountered, if you have flowering *Tradescantia*, is harvesting buds at the right stage of development. This explains why students are organized into groups, each taking a bud of different size.

Tradescantia flower buds fixed for a day in Carnoy's solution and then stored in 70% alcohol can be used for this exercise. If you have plenty of *Tradescantia* available either as a weed or in a greenhouse during the summer, we suggest killing, fixing, and storing an ample supply for later use in the laboratory.

Staining cells with aceto-carmine may be improved by using untreated or uncoated steel. This explains the use of the dissecting needle and old razor blades while staining. The anthers should be manipulated with these instruments for 2 or 3 minutes before applying the cover glass.

Heating the slides increases the intensity of the stain. This can be done by passing the slide through the flame of an alcohol lamp while making sure the slide does not get so hot that air bubbles are formed in the stain beneath the cover glass. To avoid this possibility the slide can be heated over the open mouth of a flask containing boiling water.

Slides made by this technique improve with age. To keep them from drying out, seal the cover glasses to the slides with clear nail polish and store them in a refrigerator. This will keep them semipermanent for a day or two.

Part B: Smears of Onion Root Tips

Start growing the onions at least 4 days before the topic of mitosis is to be presented to the class. The illustration (Figure 7-1-1) shows how to get the onions ready for growing root tips.

As the root grows to a length of 5 cm or more, clip off the terminal centimeter and fix it in Carnoy's solution for a day. Then store it in 70% alcohol until time to use the material in class.

The cells of onion root tips must be macerated so that they will separate and flatten when making the smear. To do this place a little 1 *M* HCl in a watch glass, cut off the terminal 3 mm of the root and put it in the HCl solution. In a matter of minutes you will notice that the root tips are soft and ready to be placed on a slide.

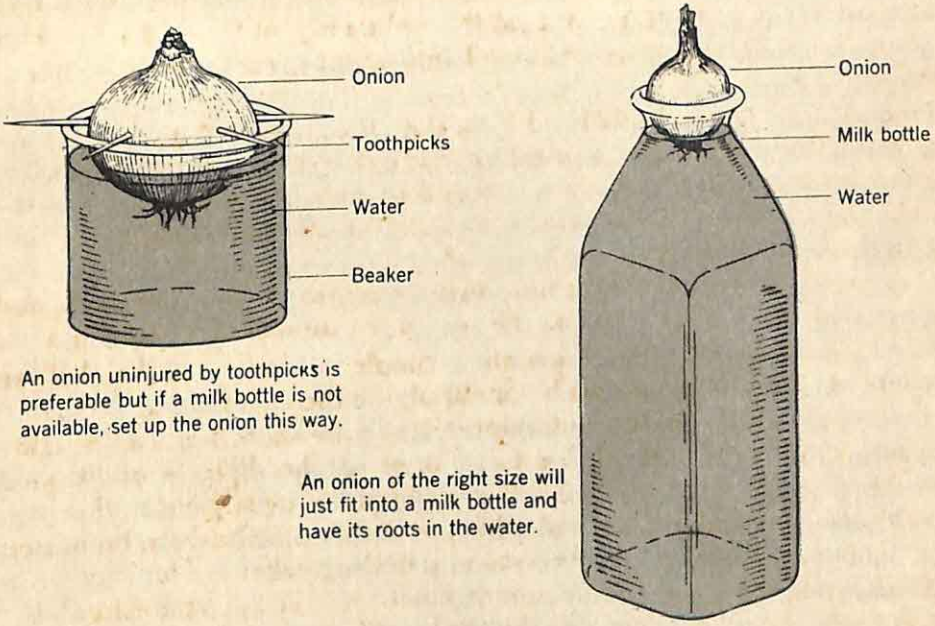
Using your forceps, take a root tip and place it directly from the HCl into a drop of aceto-carmine stain on a clean slide.

Cut the root tip into little pieces with the old, rusty razor blade.

Apply the cover glass and cover with a piece of paper towel. Then squash the root tip fragments by pushing down firmly with the eraser end of a pencil, or with the thumb, placed on top of the paper towel and cover glass. Observe under low power of the microscope. If the cells are not sufficiently separated and flattened, let the root tips stay longer in the HCl solution and also apply more pressure on the cover glass.

Part C: Nuclear and Cell Division in Onion Root Tips

Prepared slides of onion root tips are introduced because it may be impossible for you to obtain *Tradescantia* buds. If this is the case, every



An onion uninjured by toothpicks is preferable but if a milk bottle is not available, set up the onion this way.

An onion of the right size will just fit into a milk bottle and have its roots in the water.

Figure 7-1-1 Methods of growing onion root tips.

effort should be made to supplement these prepared slides with student-prepared smears of onion root tips.

Prepared slides of onion root tips are available from various biological supply houses. Be sure your slides have plenty of mitotic figures before accepting them. Those stained with hematoxylin show the structure of the spindle best.

Emphasize that the sections they are seeing are longitudinal sections of a root tip and that the root cap can be seen at the distal end. If the student is not properly oriented in relation to the section he is studying it will be difficult for him to understand the region of mitotic activity.

Part D: Animal Cells in Mitosis

Prepared slides of *Ascaris* eggs are far superior to those of whitefish but, of course, more expensive. Be sure your *Ascaris* slides show the chromosomes clearly before accepting them.

ANSWERS TO QUESTIONS

1. Six chromosomes.
2. It is obvious that there must be a replication to form the strands (daughter chromosomes), because the amount of material (DNA) in the chromosomes doubles early in cell division. It must be emphasized here that there is a *qualitative* as well as a quantitative replication. Chromosomes do not split longitudinally. If they did there would be a quantitative decrease in nuclear materials. An analogue would be a piece of chalk split down the middle. We would have two pieces of chalk, qualitatively the same, but quantitatively each one only one-half of the original piece of chalk.
3. There are two groups, 6 in each group, making a total of 12 chromosomes. One group of 6 will be incorporated into the generative nucleus of the pollen grain, the other into the tube nucleus.
4. As the student sees it, the division of the nucleus is quantitative. The qualitative replication of hereditary materials can be demonstrated only by the experimental methods of genetics.
5. The chromosomes of *Tradescantia* vary in size and form. Different students may draw different chromosomes.
6. The nucleoli have disappeared at this stage.
7. The nuclear membrane has disappeared.
8. No, 16 chromosomes cannot usually be counted because parts of them have been cut away during the sectioning process.
9. The chromosomes in onion root tips do not appear to be double with the usual methods of fixing and staining them for permanent slides.
10. Particular fibers of the spindle are attached to the centromeres of daughter chromosomes and are responsible for their separation and movement to opposite poles of the cell.
11. The new wall appears close to the midpoint of the mother cell in the center of the equatorial plate.

12. The new cell wall (cell plate) forming between two daughter cells is initiated by substances of the spindle.
13. The number of chromosomes (there are four) can easily be counted in *Ascaris* eggs, but not in those of whitefish.
14. The poles of the spindle are marked by a dark-staining body from which fibers radiate. Those fibers going toward the center of the dividing cell form the spindle. The dark-staining body is the centriole. It is absent in most plants.
15. Rather than pinching in two, which occurs in animal cells, plant cells form a new cell wall (cell plate) between the daughter cells.
16. Spindle fibers and chromosomes are present in both plant and animal cells you have studied.
17. There are no centrioles in the plant cells studied.

SUGGESTED READINGS

Books

Hughes, A. 1952. *The Mitotic Cycle.* Academic Press, New York

Levine, L. (ed.) 1963. *The Cell in Mitosis.* Academic Press, New York
Proceedings of a symposium held at Wayne State University, Detroit, November 1961.

McLeish, J. and B. Snoad. 1958. *Looking at Chromosomes.* Macmillan, London

Schrader, F. 1944. *Mitosis.* Columbia Univ. Press, New York

Swanson, C. P. 1960. *The Cell.* Ch. 4, Prentice-Hall, Englewood Cliffs, N.J.

Articles

Mazia, D. 1953. "Cell Division." *Sci. Am.* **189** [2] 53.

Swann, M. M. and J. M. Mitchison. 1958. "The Mechanism of Cleavage in Animal Cells." *Biol. Rev.* 33 p. 103.

FILMS

Smear and Squash Techniques, 1961, (sd, c, 5½ min), p or r, Thorne

Mitosis, 1961, (sd, b&w or c, 32 min), p or r, EBF

Mitosis in Endosperm, (si, b&w, 34 min), A. Bajer

Cell Biology, Part I: *Cell Reproduction*, No. 11, 1960, (sd, c, 39 min), p or r, AIBS (McGraw-Hill)

Dividing Normal Rat Fibroblasts in Tissue Culture, (si, b&w, 6 min), Carnegie

Dividing Cancer Cells Cultivated Outside the Body, (si, b&w, 8 min), Carnegie

HELPFUL BACTERIA

EXERCISE

8-1

TIME: One laboratory period.

MATERIALS: See page 67 in the *Student Laboratory Guide.*

This exercise is to accompany the nitrogen cycle as described in Chapter 8 of the textbook.

■ **The purpose of this exercise is to demonstrate that part of the nitrogen cycle dealing with the role of bacteria and the fixation of nitrogen. From this exercise the student should get some understanding of the essential role of nitrogen in plant metabolism.**

SPECIAL INSTRUCTIONS

At the beginning of the school year, if you can arrange to dig up some leguminous plants, plant them in pots and keep them growing in the laboratory until needed, it will insure a supply of material for this exercise.

A point should be made in regard to the symbiotic relationships between the two plant types in this exercise. In the symbiotic relationship between legumes and the nodule-forming bacteria, the plant supplies the bacteria with carbohydrates and the bacteria supply the plant with necessary nitrogen compounds.

This exercise introduces the student to bacteria prior to his study of microorganisms. Therefore, his observations of bacteria will be mostly perfunctory. Nevertheless, he should be able to identify the material seen in the milky nodule solution as bacteria. The emphasis is primarily on nitrogen fixation, however, and not on bacterial identification.

Part B works well as a class project or it may be done by each individual student. The students should observe the plants weekly for 3 or 4 weeks.

TECHNIQUES

Zonite is a trade name. Any other fungicide will work as well.

ANSWERS TO QUESTIONS

1. The bacterium fixes the nitrogen from the atmosphere in such a form that it eventually can be converted to plant protein.
2. The plant supplies the bacterium with carbohydrates.
3. The bacteria are not all of the same shape. They are rodlike in young nodules, but in older nodules they may have unusual shapes, becoming branched or club-shaped.

4. These bacteria cannot be considered to produce a disease in the sense that they are harmful to the plant. Rather they are helpful and this, as pointed out, is an example of mutualism. The idea here is that not all bacteria invading tissues produce a disease.
5. Because of the peculiar shapes of the nitrogen-fixing bacteria, Beijerinck was dubious as to where they fit into the normal bacterial classification.
6. Seeds inoculated with *Rhizobium* should germinate first if the species of bacterium is specific for that particular legume.
7. The inoculated legume seeds will probably produce more vigorous and rapidly growing plants.
8. The commercial inoculum, because you would then be sure you had the correct organism. In addition, preparing your own inoculum takes a great deal of time.
9. This allows both the bacterium and the plant to grow simultaneously, each supplying the required nutrient to the other and enabling the plants to grow better.
10. To remove contaminating microorganisms.
11. They increase the yield of crops. They supply the necessary nitrogen without which leguminous plants would be unable to make protein, and without plants, animals would have no protein source.

SUGGESTED READINGS

Books

Johnson, L., E. Carl, J. Bond, and H. Fribourg. 1959. *Methods for Studying Soil Microflora—Plant Disease Relationships*. Burgess, Minneapolis

Articles

Kamen, M. D. 1953. "Discoveries in Nitrogen Fixation." *Sci. Am.* **188** [3] 38

U.S. Department of Agriculture. 1953. "Legume Inoculation. What it is: What it Does." Bulletin No. 2003. U.S. Government Printing Office, Washington, D.C.

FILMS

Nitrogen Cycle, 1953, (sd, b&w, 14 min), p or r, UW

Microorganisms: *Beneficial Activities*, 1958, (sd, b&w or c, 15 min), p or r, Indiana

Microbiology, Part II: *Importance of Microorganisms*, No. 12, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Discusses decomposition, mineralization, and return of CO_2 to the air as a parallel process to the nitrogen cycle.

Nitric Acid Compounds and the Nitrogen Cycle, 1959, (sd, b&w or c, 18½ min), p, Coronet

Microorganisms

Many persons who have not had training in microbiology may believe that bacteriological techniques are extremely difficult. L. S. McClung, Indiana University, states in an article in "The American Biology Teacher," June 1960, that such is not the case and that "Practice makes perfect." In fact, most teachers, both those who have never had a course in microbiology and those who have had a little experience in microbiology would benefit by reading Dr. McClung's article and the entire issue of the publication mentioned above. Copies may be obtained by addressing a request, on your school letterhead, to:

Executive Secretary
American Society of Microbiologists
19875 Mack Ave.
Detroit 36, Michigan

Some items of equipment needed for exercises in microbiology are not normally a part of the materials available in high school biology laboratories. These include:

Inoculating loop
Inoculating needle
Wax pencil
Glass stirring rod
Roll of cotton for plugs
Cheesecloth or gauze (several 24-inch lengths)
Barnes dropping bottles. This type of bottle has a combination pipette and rubber stopper to prevent loss by spilling or evaporation. It is much less expensive than bottles with ground glass pipettes. Bottles will be needed for: distilled water, 5% phenol, crystal violet, Gram's iodine, safranin, 95% alcohol, methylene blue. One set of dropping bottles per microscope is desirable; one set per class work area is necessary. Directions for making these solutions are given in the back of this book.
Glass tumbler (to hold pencil, loop, needle, stirring rod, etc.)
Three test tubes (about 18 x 150 mm size)

Test-tube rack (One per kit is desirable. If not available, some arrangement must be made to hold the sets of tubes while filling them with agar, inoculating them, and sterilizing them.)
Wire basket (for holding groups of tubes or Petri dishes)
Funnel (especially for pouring media)

In addition to the above materials, the successful completion of the microbiology exercises requires the teacher to have available the following items of basic equipment for general class use:

Autoclave (preferred) or 16-quart pressure cooker. The small, 6-quart household-size pressure cooker is too small to sterilize test tubes of agar or 250-ml flasks.
Bunsen burners (One per student kit recommended). Propane torches may be used for flaming loops, but are too slow for preparing media. Alcohol lamps are too slow for almost anything, and are rather dangerous.
Porcelain or aluminum double boiler (to be used as a water bath). One per student kit desirable; three per class necessary.
Thermometers, −10° to 110° C, one per water bath
Two or more thermometers, 0° to 300° C, for ovens and/or incubators.
Incubator, bacteriological. Range 25° to 40° C
Drying oven or kitchen range, capable of 180° C. Combination oven-incubator with temperature range from 25° to 180° C may be used. Two are desirable so that drying will not interfere with incubation.
Refrigerator
Graduated cylinders (500 ml and 100 ml) at least one each
Double pan balance
"Sani-can" or other covered waste receptacle for waste agar and contaminated materials
Three or four 6-inch spatulas near waste can, for scraping agar out of used culture dishes
Petri dishes with covers, one per student. Presterilized plastic plates can be purchased for one-fourth the cost of Pyrex plates, but they cannot be reused.
Stock bottles of phenol and stains

An assortment of beakers and flasks is desirable, but probably can be borrowed from the chemistry department when needed.

A minimum of four work areas with water, gas, sinks, and electricity is most desirable. Less space means that the students must be in large groups or portable burners and water containers must be provided.

PREPARATION OF MEDIA AND OTHER MATERIALS

For making an agar plate, 15–20 ml agar is required. If individual tubes of agar are to be melted and used for pouring plates by each student, put that amount in separate test tubes. If the plates can be poured with agar before their use, as in the majority of the experiments, the agar may be put into flasks before sterilization and several plates subsequently poured. For example, three plates can be poured from a 125-ml flask containing 50 ml agar.

Sterilize all media in the autoclave or pressure cooker at 15 lbs pressure for 15 minutes. Directions for using the autoclave or pressure cooker should be available from the manufacturer. If instructions are not available, consult a home demonstration agent or any home economics specialist.

Sterilize the Petri dishes in a hot air oven at 175° C for 1 hour.

Wrap pipettes in paper, and sterilize them in the autoclave.

CLEAN-UP PROCEDURE

Disinfecting solutions for general laboratory use are (a) 1% phenol, or 2% Lysol, or 2% Clorox; (b) 5% phenol. Make up liter quantities. Mark the bottles *POISON*.

Laboratory work areas should be cleaned and disinfected with solution at the beginning and the end of the laboratory period. Students should wash their hands immediately after using the disinfecting solutions.

When microbial cultures are accidently broken or contents spilled, pour 5% phenol liberally over them and allow it to stand 10 minutes to kill the microorganisms *before* cleaning up.

To prevent gross contamination of the laboratory and the preparation room, all materials after use should be sterilized in the pressure cooker or autoclaved at 15 lbs pressure for 15 minutes before discarding or washing.

All glassware should be thoroughly washed with a good detergent in warm water. Drain and dry, and return to storage place.

MICROBIOLOGICAL TECHNIQUES

EXERCISE 9-1

TIME: One laboratory period.

MATERIALS: See page 71 in the *Student Laboratory Guide.*

This exercise is not keyed to a specific text chapter but should be done before proceeding with other microbiological exercises. It will undoubtedly be best done while the viruses are being studied in Chapter 9, as there are no specific laboratory exercises dealing with viruses.

■ **The purpose of the exercise is to acquaint the students with the techniques of handling microorganisms, so that they will be able to understand the problems involved in dealing with microorganisms and be able to perform the subsequent work with sufficient accuracy to obtain the desired results.**

SPECIAL INSTRUCTIONS

This exercise should be assigned as homework and should be read before coming to the demonstration. It is possible to answer all of the questions contained in the exercise prior to the demonstration, but they will be more meaningful if answered afterwards.

With proper previous preparation this demonstration can be done in one classroom period. However, it is essential that the students thoroughly understand the techniques of handling microorganisms. Sufficient time should be spent on this exercise for the students to become proficient in streaking plates, pouring agar, and in general, preparing for actually handling cultures. Failure to master the rudiments of technique at this stage will make the succeeding exercises on microorganisms much more difficult.

TECHNIQUES

If the students read and understand the exercise before coming to class, the ideal introduction is the showing of the BSCS high school biology technique film entitled "Bacteriological Techniques." This short film can be shown several times during the period if necessary for reinforcement of points involved in the techniques.

After showing the film it will be advantageous to demonstrate to the students what they have seen in the film concerning transfers, streaking, preparation of media, rolling plugs, and handling microorganisms.

If time allows, students can be given the opportunity to roll cotton plugs, practice the technique of flaming loops and mouths of test tubes, to prepare media, pour plates, and slants, and to practice transferring and streaking, preferably with water solutions.

It is by observing actual student practice that you will find whether or not it is necessary either to show the technique film again or to demonstrate specific techniques to students who have not yet attained proficiency.

ANSWERS TO QUESTIONS

1. It would be more likely to inhibit bacterial growth.
2. Because it would not be as strong a solution as a disinfectant.
3. (a) From the surrounding air; (b) from contamination of the transfer needle or loop; (c) by accidentally touching some contaminated object with the needle or loop during transfer.
4. (a) By exposing the dishes and cultures as little as possible to the outside air, and keeping them covered at all other times; (b) by proper sterilization of loops and needles before making transfers; (c) by developing a steady hand so the loop and needle do not touch the sides of tubes or other objects.
5. In a pure culture you can deal with a single organism and properly interpret the results. In a mixture of microorganisms you would not be sure which organism was chiefly responsible for each of the observed results.
6. Corks would not allow passage of filtered air. Cork is not as easily disposable. A cork is not sterilizable by flaming. Various sizes of corks would have to be stocked for various sizes of tubes. The cork does not usually fit smoothly and tightly, and contamination often takes place.
7. There has been contamination of Petri dish B.
8. It would be a worse culture medium.
9. Because exposure to so high a temperature (98° C) would kill most microorganisms.
10. Pour a suitable disinfectant over the area and allow it to stand 10 minutes. Carefully sweep up the broken glass and agar and discard them in an appropriate container. Wash the area with soap and water to remove the disinfectant. Thoroughly wash hands.

SUGGESTED READINGS

Books

Beck, J., D. Larsen, D. Donaldson, and R. Sayers. 1960. *Laboratory Manual for General Microbiology.* Burgess, Minneapolis
Burrows, W. 1963. *Textbook of Microbiology.* 18th ed. W. B. Saunders, Philadelphia
Schoenhard, D. 1961. *Basic Concepts and Experiments in Microbiology.* Burgess, Minneapolis
Sussman, A. 1961. *Biology Through Microbes.* Univ. of Michigan, Ann Arbor
Wedburg, S. 1963. *Paramedical Microbiology.* Reinhold, New York
Wyss, O., O. B. Williams, and E. W. Gardner. 1963. *Elementary Microbiology.* Wiley, New York

FILMS

Bacteriological Techniques, 1960, (sd, c, 5 min), p or r, Thorne
Bacteria: *Laboratory Study*, 1958, (sd, b&w or c, 15 min), p or r, Indiana

A GARDEN OF MICROORGANISMS

EXERCISE 9-2

TIME: One laboratory period. A 10- to 15-minute observation period on several subsequent days.

MATERIALS: See page 77 in the *Student Laboratory Guide*.

This exercise should be performed while doing Chapter 9 in the textbook. It anticipates the work in the later chapters by introducing a wide variety of microorganisms including molds, bacteria, protozoans, algae, and yeasts.

■ **The purpose of the exercise is to impress the students with the wide range of microorganisms and with the fact that microorganisms compete with other organisms for survival. This "garden" will demonstrate that microorganisms are virtually everywhere and that they do not spoil foods to spite man but do so to maintain their own existence. The student will have the opportunity to recognize colonies of microorganisms both macroscopically and as individual microorganisms under the microscope.**

SPECIAL INSTRUCTIONS

It is important that all equipment used should be clean to start with and should be cleaned carefully after the study is completed. For successful growth, chemical cleanliness (absence of soap, detergents, etc.) is in this exercise more important than sterility. This means rinsing glassware thoroughly at least five times after washing.

This experiment may be used as a demonstration. Large dishes (1000 ml) are necessary if but one set is used for all classes. If smaller dishes are used, with proportionately less material, there will be correspondingly less growth for study.

Students should be involved in the preparation for this exercise and be asked to bring in different sorts of food and other organic materials. It is suggested that each team be instructed to place about the same amount of material in the dishes, since the final analyses will then be more comparable. Each team should keep daily records of their "garden of microorganisms" and later report their findings on a master record sheet with those of the other teams concerned.

A 10- to 15-minute observation time will be necessary on each of several subsequent days.

TECHNIQUES

Peppercorns can be purchased in any large grocery store.

Dried grass may be substituted for the hay.

The containers used can be of any transparent type. They do not have to be stacked, but the desirability of conserving space is very important. Do not place the stack of dishes in direct sunlight or over a radiator. After preliminary study of the microscopic structures, keep the cultures going to supply organisms for later exercises.

In some areas there may be a problem because of chlorine in the water. To eliminate the chlorine the following is suggested: Place a small amount of photographic hypo (sodium hypochlorite) in a liter of water and allow it to stand an hour. Usually a single crystal of hypo is sufficient. Place the water in an open container and allow it to stand for 24 hours before using.

Some of the microorganisms will produce an unpleasant odor. For this reason a ventilated area for storage is desirable, and some of the containers should be opened and examined only with good ventilation.

To obtain the best results, divide the class into groups or teams and let each team set up its own garden of microorganisms. The number of students per team and the number of dishes will be determined by the amount of equipment available.

Most of the cultures produced will last for several weeks, except the protozoans. These must have some nutrient added to the dish weekly. Powdered milk, Ceraphyl, or dried lettuce does an excellent job in maintaining protozoan cultures. A small amount of any of these added to the culture should keep it active for several weeks.

The student should be able to ascertain the presence of molds, bacteria, protozoans, algae, and yeasts in this garden. The garden may be altered to maintain or obtain many different types of microorganisms. It is sufficient for the purpose of this exercise that the students recognize only the major categories, for subsequent exercises will deal with each in greater detail.

ANSWERS TO QUESTIONS

1. through 6. are to be determined by the student on the basis of classroom observations of the garden of microorganisms.
7. The microorganism growth will not look the same in each dish. Molds

and algae will have a different appearance from each other and will differ from the bacteria, protozoans, and yeast.

8. The student will be able to see molds and clusters of algal cells with the unaided eye. Bacteria and yeasts will not be seen as individuals but colonies will be noticed. Protozoans will not be seen easily as individuals but will cloud the culture.
9. Even though the microorganisms cannot be seen, the results of their metabolism are noticeable in their effects on the various foodstuffs used.
10. Samples of growths are not similar in appearance.
11. The student should recognize algae and protozoans from having seen them earlier in the pond-water exercise.
12. Growth of microorganisms could be hindered or prevented by (a) excluding contaminated air, (b) thoroughly sterilizing the material used, and (c) by sterile techniques in handling.

SUGGESTED READINGS

Books

Kelly, F. C. and K. E. Hite. 1955. *Microbiology.* Appleton-Century-Crofts, New York

Pelczar, M. J. and R. D. Reid. 1958. *Microbiology.* McGraw-Hill, New York

FILMS

Microbiology, Part II: *The Very Small,* No. 1, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Microbiology, Part II: *Microorganisms with Chlorophyll,* No. 4, 1961, (sd, c, 32 min), p or r, AIBS(McGraw-Hill)

STAINING AND OBSERVING BACTERIAL CELLS

EXERCISE 10-1

TIME: One laboratory period.

MATERIALS: See page 79 in the *Student Laboratory Guide.*

This exercise is to accompany the section on bacteria, in Chapter 10 of the textbook.

■ **The purpose of this exercise is to acquaint the student with the techniques used in staining and observing the characteristics of bacteria taken from a peppercorn infusion.**

SPECIAL INSTRUCTIONS

It is important that a very thin film of the peppercorn infusion be prepared. If film is too thick, the staining and observation will be difficult.

Caution against using more than one or two drops of stain. Make sure that a *gentle* rinsing of the slide is done; otherwise the film will be removed from the slide.

TECHNIQUES

Yogurt is also an excellent source of bacteria. It shows both the cocci and bacilli in great quantity.

Because of the small number of oil immersion objectives usually available, the oil immersion observation may have to be done as a demonstration.

ANSWERS TO QUESTIONS

1. Bacteria appear to be tiny dots or spots. The student will probably be unable to make out any particular detail.
2. The student should just be able to determine the shapes of individual bacteria.
3. In their shapes, whether they are round, rod-shaped, or spiral and whether they are single or joined in clusters, chains, or pairs.
4. Bacilli also form chains and clusters, like cocci.
5. This will depend on the bacteria being observed. The largest forms are usually the bacilli and the smallest the cocci. A typical bacillus may measure 1.0 to 1.3 x 3 to 10 μ in size. An exceptional case is found in the cockroach intestine, where a bacillus (*Bacillus bütschlii*) grows as large as 0.6 x 80 μ. The size range of cocci is from 0.4 to 0.8 x 0.8 to 1.0 μ. Spirilla are usually relatively large.
6. Typical diseases are as follows: (a) cocci: streptococcus throat infection, scarlet fever, rheumatic fever, childbirth (puerperal) fever, pneumococcus infections such as pneumonia, gonococcus infection causing gonorrhea, meningitis; (b) bacilli: dysentery, typhoid, paratyphoid, diphtheria, tuberculosis, tetanus, tularemia, and botulism; (c) spirilla: cholera, relapsing fever, and syphilis.

SUGGESTED READINGS

Books

Burrows, W. 1963. *Textbook of Microbiology.* 18th ed. W. B. Saunders, Philadelphia

Clark, F., and O. Halvorson. 1958. *Laboratory Outline for General Bacteriology.* Burgess, Minneapolis

Thimann, K. 1955. *The Life of the Bacteria.* Macmillan, New York

Articles

Morowitz, H. J. and M. E. Tourtellotte. 1962. "The Smallest Living Cells." *Sci. Am.* **206** [3] 117

FILMS

Microbiology, Part II: *Bacteria*, No. 8, 1961, (sd, c, 31 min), p or r, AIBS(McGraw-Hill)

Microorganisms: *Harmful Activities*, 1958, (sd, b&w or c, 15 min), p or r, Indiana

DISTRIBUTION OF MICROORGANISMS

EXERCISE

10-2

TIME: One period for preparation, and observation times on each of 3 or 4 subsequent days.

MATERIALS: See page 81 in the *Student Laboratory Guide.*

This exercise is to accompany Chapter 10, which has an introductory portion on the distribution of microorganisms.

■ **The purpose of this exercise is to demonstrate the wide distribution of microorganisms in our immediate environment and to suggest ways for reducing their numbers.**

SPECIAL INSTRUCTIONS

This exercise is concerned with the distribution of microorganisms over surfaces. Be sure to have some students test various cleaned surfaces as well as dirty surfaces.

Considerable time can be saved if the medium is prepared and poured into sterilized Petri dishes before the exercise is to be done.

TECHNIQUES

Variations of the transfer technique can be used. If sufficient dishes are available, a separate Petri dish can be used for each piece of tape.

Masking tape can be substituted for cellophane tape.

Many teachers prefer individual rolls of cellophane tape for individual student groups.

As an alternative for the technique of transferring microorganisms by cellophane tape, the contact-dish technique might prove interesting.

For each pair of students the contact-dish technique requires the following:

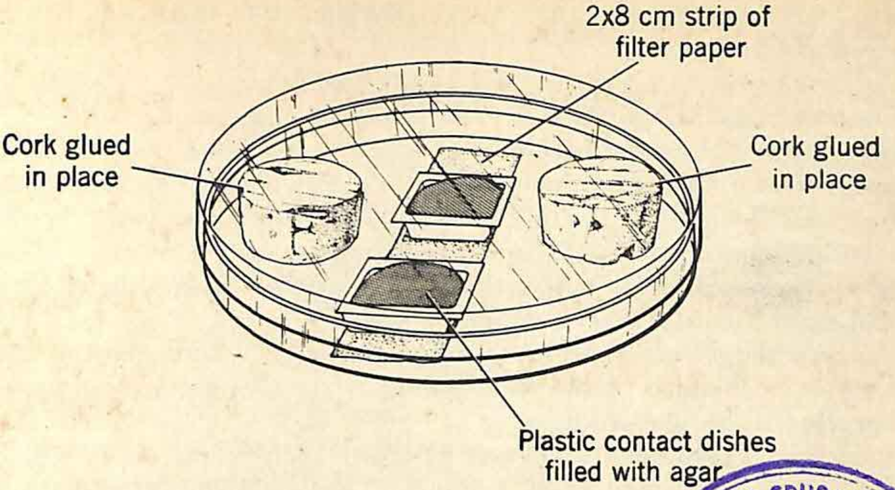

2x8 cm strip of filter paper
Cork glued in place
Cork glued in place
Plastic contact dishes filled with agar

Figure 10-2-1 **Petri dish incubation chamber**

STATE INSTITUTE OF EDUCATION
Govt of West Bengal

Two plastic jelly dishes (similar to those used for individual servings of jelly in restaurants)
40 ml of nutrient agar in a flask
One clean Petri dish
One No. 5 cork stopper
One strip of filter paper 2 x 8 cm
Rubber cement
Water bath
Thermometer
Forceps
Jar of 70% alcohol
Paper towel

The technique is as follows:

Preliminary Preparations

Put plastic contact dishes into a jar of 70% alcohol overnight to disinfect them.

Prepare nutrient agar. Put 40 ml into a 125-ml flask. Sterilize.

A Petri dish makes a good incubation chamber for the contact dishes (see Figure 10-2-1). It may be necessary to raise the cover of the dish to prevent it from touching the agar after it has solidified in the contact dishes. Two halves of a No. 5 cork stopper no longer than 2 cm will support the Petri dish cover. Place the two halves of cork opposite each other just inside the rim of the bottom half of the Petri dish. Fasten the cork supports with rubber cement. Put a strip of filter paper in the bottom of the dish and moisten with a few drops of water. Replace the cover of the Petri dish.

Preparation of Contact Dish

With forceps that have been in 70% alcohol for at least 5 minutes, remove a plastic dish from the jar of alcohol. Drain on a paper towel. Then place in the incubation chamber. Repeat for a second dish.

Pour melted agar cooled to 50° C (agar can be melted in a boiling water bath while the incubation chamber is being made) into the two plastic dishes until they are full almost to overflowing and the agar extends above the rim of the dishes.

Replace the Petri dish cover and let the agar gel.

After the agar has solidified, touch the agar of the contact dish to any surface in the classroom, laboratory, lavatory, cafeteria, water fountain, shower room floor, food preparation surfaces in the home economics laboratory, etc. Cooking and eating utensils may also be touched. The other dish, which has no contact, should be maintained as a control.

After the imprints have been made, replace the contact dishes in the chamber for incubation at room temperature.

Examine the dishes daily for the appearance of microorganism growth in the form of colonies. Make final observations after three or four days. Count the number of colonies.

Time and energy can be saved by using ready-made contact dishes. These can be purchased as "Rodac surface contact plates" from Baltimore Biological Laboratories, 2201 Aisquith St., Baltimore 18, Md.

Some suggested forms for reporting individual and class results appear at the end of this exercise.

ANSWERS TO QUESTIONS

1. Microorganisms are present on practically all surfaces.
2. Air-borne microorganisms and microorganisms transferred by contact easily contaminate surfaces.
3. The question of relative abundance is difficult to establish. There are undoubtedly more bacteria than molds. However, because the molds grow readily and rapidly they may obscure the presence of other microorganisms.
4. Microorganisms from an infected individual can become established on a surface and can be picked up on the hands of a second individual and subsequently transferred to the mouth.
5. By cleaning surfaces, by refraining from touching more surface areas than necessary, by observing personal habits of cleanliness such as washing hands.

SUGGESTED READINGS

Books

Dubos, R. J. 1958. *Bacterial and Mycotic Infections of Man.* Lippincott, Philadelphia

Grant, M. P. 1953. *Microbiology and Human Progress.* Holt, Rinehart and Winston, New York

FILMS

Microorganisms: *Harmful Activities*, 1958, (sd, b&w or c, 15 min), p or r, Indiana

Microbiology, Part II: *The Germ Theory of Disease*, No. 10, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

CHART FOR MICROORGANISM IMPRINTS

Quadrant No. of Dish	Sources of Microorganisms	Number of Colonies								Remarks
		Bacteria Days				*Molds Days*				
		1	*2*	*3*	*4*	*1*	*2*	*3*	*4*	
1										
2										
3										
4	*(Control)*									

SAMPLE HEADINGS FOR MASTER CHART OF CLASS RESULTS

Student Name	Source of Microorganisms	Total Number of Colonies		Remarks
		Bacteria	*Molds*	

PURE CULTURES OF MICROORGANISMS

EXERCISE 10-3

TIME: Two days of preparation (can be reduced to 1 if preparation is done before class) plus observation periods for each of 3 succeeding days.

MATERIALS: See page 83 in the *Student Laboratory Guide.*

This exercise is to accompany Chapter 10 of the textbook and will demonstrate the techniques of obtaining the pure cultures used in experiments described in this chapter.

■ The purpose of this exercise is to acquaint the student with dilution techniques and their value in estimating numbers of microorganisms as well as their value in obtaining pure cultures of microorganisms by either the streak-plate or pour-plate method.

SPECIAL INSTRUCTIONS

The amounts of materials given in the *Student Laboratory Guide* are predicated on groups containing ten students. Materials can be augmented or reduced as necessary, depending upon the number of students to be served and the equipment available. The inoculating loops should be of 0.05-ml capacity.

A day will be saved if the preparation of the culture medium is done before classes begin.

Most of the cultures required here have been used in earlier exercises, particularly 9-2. The mechanics of dilution should be clearly understood in order for the students to calculate the numbers of microorganisms in their cultures.

In order to preserve the laboratory guides intact, the chart of observations and the chart for the numbers of bacterial colonies can be copied by the students and handed in individually.

It may be well to have the students review the earlier exercise (6-6) involving dilutions.

TECHNIQUES

Mixed suspensions of bacteria other than those suggested can be used for this experiment.

A 1-cm grid is easily prepared by ruling a sheet of paper into 1-cm squares over which the Petri dishes can be placed.

Of the two methods used in preparing plate cultures, probably the most difficulty will be experienced with the pour-plate method, since there is a tendency to let the agar cool below 45° C. It then becomes difficult to pour and forms lumps.

Remind the students to cool the loops before attempting to transfer the bacteria. A review of the procedures learned in Exercise 9-1 would help.

You may wish to demonstrate again certain techniques, such as sterilizing forceps and bacteriological loops in a flame, making transfers to Petri dishes, transferring from tube to tube, and plating.

It will be particularly valuable to demonstrate again the technique of streaking and perhaps to show again the BSCS film on bacteriological techniques.

Cultures of microorganisms can be obtained from several supply houses, or from the American Type Culture Collection, 2029 M St., N.W., Washington 6, D.C. Some university and college departments will willingly supply bacterial cultures if the demands do not become too heavy.

ANSWERS TO QUESTIONS

1. 1:10 (10^{-1} or 0.1)
2. 1:100 (100^{-2} or 0.01)
3. Ten times

4. 1 : 1000 (10^{-3} or 0.001)
5. Ten times
6. One hundred times
7. Two loops equal 0.1 ml and therefore there would be a 1 : 100 dilution if you added two loops of source material to 9.9 ml sterile water. Therefore, the loop dilutions are not identical with the 1.0 ml dilutions.
8. No. The ratios would be different after the initial inoculation. One ml transfers give a ratio of 10 (1 : 10, 1 : 100, 1 : 1000). The 0.1 ml transfers give a ratio of 100 (1 : 100, 1 : 10,000, 1 : 1,000,000).
9. and 10. Answers depend upon student results.
11. The effect of dilution on each of the sources is to reduce the number of colonies present.
12. Yes. This can be calculated from the counts at 1 : 1000 dilution.
13. From student observation, but most likely the bacterial population.
14. From student data.
15. From student data. The streaking procedure, however, is superior for isolation of aerobes.
16. The answer will depend on the count from the plates, and it is derived in the following fashion. Count the number of colonies in one square centimeter. Determine the diameter of the dish in centimeters. $\pi (d/2)^2$ [πr^2] will give you the area of the dish. The area of the dish times the count of colonies in one square centimeter will give you the approximate number of bacterial colonies on the dish.
17. The number of colonies on the dish (two loops equal 0.1 ml) times 10 will equal the number of bacteria per milliliter.
18. The number per milliliter in Tube 1 times 10 will give the concentration per milliliter of source material. The number per milliliter in Tube 2 times 100 will also give the concentration per milliliter of source material.
19. Student observation.
20. If organisms were present in the control, it indicates inadequate technique.

SUGGESTED READINGS

Books

Kelly, F. C. and K. E. Hite, 1955. *Microbiology*. Appleton-Century-Crofts, New York

Peltier, G., C. George, and L. Lindgren. 1952. *Laboratory Manual for General Bacteriology*. Wiley, New York

Articles

American Society of Microbiologists. 1960. "Microbiology in Introductory Biology." *American Biology Teacher*, **22** [6] June

Available from the Executive Secretary, American Society of Microbiologists, 19875 Mack Ave., Detroit 36, Mich.

FILMS

Bacteria: *Laboratory Study*, 1958, (sd, b&w or c, 15 min), p or r, Indiana

Bacteriological Techniques, 1960, (sd, c, 5 min), p or r, Thorne

ANTIBIOTICS AND BACTERIA

EXERCISE

11-1

TIME: One laboratory period plus a second observation and data-recording time.

MATERIALS: See page 87 in the *Student Laboratory Guide.*

The students should be referred to Chapter 11 of the textbook for a discussion of antibiotics.

■ **The purpose of the exercise is to demonstrate in a quantitative fashion the effects of various antibiotics on different bacteria.**

SPECIAL INSTRUCTIONS

In the list of materials, only the active principles contained in antibiotics are named. Different trade-name antibiotics frequently contain the same active principle, for example, tetracycline, which is an ingredient of several different trade-name antibiotics. The antibiotics named here are generic and are marketed under a number of different trade names.

"Invitations to Enquiry," No. 16, "Discovery of Penicillin" in the *Biology Teachers' Handbook* demonstrates accident in enquiry and will be useful at this point.

TECHNIQUES

This exercise lends itself well to dividing the class into teams.

If preferred, plates can be divided into quarters or into sixths, depending on how many antibiotic disks you wish to use. However, if the students are inexperienced, the division into thirds is about all they can handle in one period.

If you wish to insure an excellent bacterial growth before applying antibiotic disks, let 36 hours elapse before inoculating the culture plates with the antibiotic disks.

Substitutions can be made—for example, *Staphylococcus aureus* can be substituted for *Staphylococcus albus* or *Bacillus subtilis* can be substituted for *Staphylococcus albus.*

Students should be cautioned not to break the agar surface when streaking. Some teachers have found a cotton swab an excellent device for streaking rather than an inoculating loop.

Emphasize in student discussions that not all antibiotics are equally effective with each type of microorganism and lead from this to a discussion of antibiotic specificity.

ANSWERS TO QUESTIONS

1. and 2. Depends on the microorganism and the antibiotic used, but both organisms should not be equally sensitive to every antibiotic used.
3. and 4. Depends on which antibiotic is used, although penicillin is not as effective on *E. coli* as some of the other antibiotics would be. The tetracyclines tend to be quite effective on *E. coli.*
5. Any antibiotic which destroys the intestinal flora will cause some intestinal disturbances and in many cases the intestinal flora will need to be replaced.
6. That there is a certain antibiotic specificity but that some antibiotics, in addition to destroying the harmful bacteria, may upset the normal bacterial balance within the body.
7. From this experiment it is possible to compare the clear areas around the various antibiotics. However, this experiment lacks a control, as the next question brings out.
8. This experiment has no control.
9. A disk containing no antibiotics should be used as a control.

SUGGESTED READINGS

Books

Cooley, D. 1954. *The Science Book of Wonder Drugs.* Pocket Books

Spector, W. S. 1957. *Handbook of Toxicology: Vol. 2, Antibiotics.* W. B. Saunders, Philadelphia

Articles

Gray, G. W. 1949. "The Antibiotics." *Sci. Am.* **181** [2] 26

Pramer, D. 1955. "Antibiotics Against Plant Diseases." *Sci. Am.* **192** [6] 82

Rane, L. and Dora S. Rane. 1949. "Aureomycin." *Sci. Am.* **180** [4] 18

Raper, K. B. 1952. "The Progress of Antibiotics." *Sci. Am.* **186** [4] 49

FILMS

Antibiotics, 1952, (sd, b&w or c, 14 min), p or r, EBF

And the Earth Shall Give Back Life, 1952, (sd, b&w, 25 min), free loan, Squibb

TEMPERATURE AND MICROORGANISMS

EXERCISE

11-2

TIME: One laboratory period plus several short periods of observation subsequent to the exercise.

MATERIALS: See page 89 in the *Student Laboratory Guide.*

This exercise is to accompany the section concerned with control of spoilage and pasteurization, in Chapter 11 of the textbook.

■ **The purpose of this exercise is to demonstrate the effect of temperature on the growth of microorganisms and to demonstrate ways in which temperature control is used to prevent spoilage.**

SPECIAL INSTRUCTIONS

If it is impossible to secure raw skim milk in your area, either pasteurized skim milk inoculated with 1% buttermilk, or rehydrated powdered milk with 1% buttermilk may be substituted. The heating of the two sets of test tubes demonstrates two methods of pasteurization. The 62° C temperature represents the low-temperature-holding (LTH) method of pasteurization. The 90° C temperature represents the high-temperature-short time (HTST) method of pasteurization. Actually the temperature recommended here is higher and the time is longer than those customarily used. Usually, the temperature of the HTST is 70° to 72° C for 17 to 19 seconds.

TECHNIQUES

Alter the student group size according to available equipment and size of class, having each student do a specific part. This saves considerable time and allows you to check on the individual student. The materials listed in the *Student Laboratory Guide* are for the entire class.

This exercise can be done without the refrigerator and the incubator if there are areas of standard but different temperatures available in which the tubes can be placed.

Much time can be saved if two water baths are available, one at 62° C, the other at 90° C.

As the results are to be recorded in chart form, it is desirable in order to obtain uniform results and for ease of checking, to place the desired format on the blackboard before the beginning of the exercise.

Emphasize the critical aspect of the temperature relationship in this exercise.

ANSWERS TO QUESTIONS

1. Bacteria.
2. In the unpasteurized tubes stored at room temperature. The answer to the rest of the question depends on the student observations, but in general the nonheated tubes will have a greater growth than the heated ones and those nearest room temperature will have the greatest amount of growth in comparison to those at higher or lower temperatures.
3. Because it kills the common, nonspore-forming bacteria without altering the taste or quality of the milk.

4. At low temperatures corresponding to those of a refrigerator.
5. Nonspore-formers are killed, both by the 62° C and by the 90° C heating as well as by incubation at temperatures of 45° C and above. The optimum range for most organisms is below 45° C which is a maximum for organisms adapted to the middle ranges of temperature.
6. Curdling takes place soonest at room temperature or 23° C. Cultures in the refrigerator will eventually curdle, but it may take them several days or even weeks. Curdling will be slow in the 45° C incubator. Therefore, microorganisms in milk grow best at room temperature or at 23° C but are slowed in growth at refrigerator temperatures or at temperatures of 45° C or above. Heating to higher temperatures destroys the nonspore-forming bacteria.

SUGGESTED READINGS

Books

Burrows, W. 1963. *Textbook of Microbiology*, 18th ed. W. B. Saunders, Philadelphia
Chapter 12 deals with the microbiology of milk and food.
Chalmers, C. H. 1955. *Bacteria in Relation to Milk Supply*. Arnold, London
Davis, J. G. 1955. *Milk Testing*. Dairy Institutes Ltd., London

Articles

Tittsler, R. P., *et al.* 1952. "Symposium on Lactic Acid Bacteria." *Bacteriol. Rev.* **16** p. 227

FILMS

Milk, 1956, (sd, b&w, 11 min), p or r, EBF

DIGESTION OF FOODS BY MICROORGANISMS

EXERCISE 11-3

TIME: Parts A and B: 1 day of student participation; 2 to 4 days of incubation plus an observation and recording period.

MATERIALS: See page 91 in the *Student Laboratory Guide.*

This exercise is to accompany the portion on microbial nutrition and enzymes in Chapter 11 of the textbook.

■ **The purpose of this exercise is to indicate carbohydrate and protein digestion by various microorganisms and to compare the nutritional requirements of microorganisms.**

SPECIAL INSTRUCTIONS

Pay particular attention to the dishes during the incubation period, for frequently there will be a very rapid growth, particularly of molds, in the starch dish.

TECHNIQUES

This exercise can be best done by teams of students. The hay infusion, yeast suspension, and mold suspension from Exercise 9-2 can be used here.

S. lutea can be substituted for *B. subtilis.*

Students should be cautioned to remember to flame and cool the loops before making their streak cultures.

ANSWERS TO QUESTIONS

1. Depends on student results, but the hay, the yeast, and the mold should all have clear areas around them.
2. The clear area indicates that enzymes are present for the digestion of starch to sugar.
3. The absence of a clear area means the organisms do not have enzymes that digest starch.
4. Starch digestion is probably most extensive with the molds, next most extensive with the yeasts, and least with the bacteria of the hay infusion. All three organisms contain starch-digesting enzymes.
5. They should be free of microorganisms. If microorganisms are present, question the techniques used.
6. All three sets of organisms will grow, but not as well on the starch-free plate as on the starch plate.
7. Bacteria and yeasts will probably do better on the nonstarch plate than will molds.
8. The microorganism contains an enzyme that digests protein.
9. Depends on student results, but normally one expects the protein digestion to be more extensive, particularly as the bacteria in a hay infusion subsist largely on protein.
10. The protein digestion would take place more rapidly than the carbohydrate digestion in the case of the hay infusion.

SUGGESTED READINGS

Books

Hawker, L. E., *et al.* 1960. *An Introduction to the Biology of Microorganisms.* Arnold, London

Chapter 9 deals with microorganism nutrition.

FILMS

Microbiology, Part II: *Metabolic Diversity*, No. 11, 1961, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

Cell Biology, Part I: *Growth and Replacement*, No. 9, 1960, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)

BACTERIAL POPULATIONS IN MILK

EXERCISE 11-4

TIME: One laboratory period, with subsequent observation time during the following 8 hours.

MATERIALS: See page 93 in the *Student Laboratory Guide.*

This exercise is to accompany the section on bacterial growth and pasteurization in Chapter 11 of the textbook.

■ **The purpose of this exercise is to demonstrate to the students a test for bacteria based on the oxygen consumption of bacteria in various samples of milk.**

SPECIAL INSTRUCTIONS

Students will be able to read the results from badly contaminated and poorly rated milk within the classroom period. However, the more bacteria-free the milk, the longer the tests will take. Therefore, it may be possible to assign students to observe the tests throughout the day to record the decolorization time of the milk of better quality.

The decolorizing can be considerably slowed by putting the samples in a refrigerator and taking them out on a subsequent day before class. It is possible to delay decolorization in pasteurized milk samples for periods of up to 4 days.

"Invitations to Enquiry," No. 31, in the *Biology Teachers' Handbook* uses population growth in bacteria to explain exponential functions and may be useful here.

TECHNIQUES

Since this exercise works well with student teams, the materials listed in the *Student Laboratory Guide* are per team.

You may wish to introduce the concept of a control into this experiment by using uninoculated methylene blue solutions and comparing the results.

ANSWERS TO QUESTIONS

1. Depends upon student observation.
2. The older the milk sample, the higher the count for similar types of milk.
3. Cow not properly cleaned before milking; dirty barn or stall; dirty milk

pails or machines; dirty hands of human attendant; dirty milk cans or storage cans and trucks; unsanitary containers after pasteurization; high storage temperatures after pasteurization, either at dairy, in delivery truck, or at home.

4. Strict control of the sanitary conditions at the dairy farm, the dairy and during delivery. Public education, so that milk will be properly refrigerated at home.
5. Milk stored at higher temperatures will contain more bacteria and will decolorize methylene blue in a shorter time than the same sample stored at lower temperatures. Therefore bacteria multiply more rapidly at room temperatures than at low temperatures.
6. A high bacterial count in pasteurized milk indicates that there was either a very high count before pasteurization or that the milk was not refrigerated at a low enough temperature after pasteurization.

SUGGESTED READINGS AND FILMS

See Exercise 11-2

SUGAR FERMENTATION BY YEASTS AND BACTERIA

EXERCISE

11-5

TIME: Preparatory period, 1 laboratory period for student participation, and 2 or 3 days of observations of incubated cultures with a subsequent time for recording observations and answering questions.

MATERIALS: See page 95 in the *Student Laboratory Guide.*

This exercise should accompany the discussion of aerobic and anaerobic respiration in Chapter 11 of the textbook.

■ **The purpose of this exercise is to compare and to contrast the fermentation by yeasts and by bacteria of various sugar substrates and to demonstrate the nature of anaerobic metabolism.**

SPECIAL INSTRUCTIONS

The Durham fermentation tubes should be prepared before class time and should be sterilized before use.

The students are asked to make a bar graph showing the amount of gas produced in each of the eight tubes per student team. A discussion of bar graphs should precede this request.

"Invitations to Enquiry," No. 28, in the *Biology Teachers' Handbook* deals with change of rate through a discussion of rate of fermentation and may be introduced here.

You may wish to refer students to Figure 4-4 on page 71 in the textbook as an example of a simple bar graph which can be contrasted with the complex bar graph, Figure 39-9, on page 701 in the textbook.

TECHNIQUES

The materials listed in the *Student Laboratory Guide* are for a class of 32 but may be reduced according to the number of students in each team.

Bromthymol blue is blue at pH 7.6, yellow at pH 6 and thus is a test of pH from approximate neutrality into the acidic range.

A 0.04% solution of bromthymol blue is sufficient for this exercise.

ANSWERS TO QUESTIONS

1. Carbon dioxide.
2. By bubbling it through limewater. A milky color forms in the presence of CO_2.
3. Both aerobic and anaerobic metabolism.
4. That the solution becomes acidic.
5. Carbon dioxide reacting with water to form carbonic acid, which ionizes into H^+ and carbonate ($CO_3^=$) or bicarbonate (HCO_3^-) ions.
6. Characteristic of both aerobic and anaerobic metabolism. The difference is that in the experiment no O_2 was available from the air for the metabolism of the microorganisms.
7. Because anaerobic metabolism results in alcohol plus CO_2, while the end products of aerobic metabolism are water and CO_2.
8. Based on student observation. Usually the yeast tubes.
9. Student observation, but usually bacteria.
10. Probably the yeast.
11. Glucose.
12. No reaction should be noticed at all in the control tubes if proper sterile techniques have been observed. If there is a reaction in the controls then the technique is suspect.

SUGGESTED READINGS

Books

Burrows, W. 1963. *Textbook of Microbiology.* 18th ed. W. B. Saunders, Philadelphia
Chapter 5 deals with bacterial metabolism.

Downes, H. R. 1962. *The Chemistry of Living Cells.* Harper & Row, New York
Chapter 15 deals with anaerobic carbohydrate metabolism.

FILMS

Microbiology, Part II: *Metabolic Diversity*, No. 11, 1961, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

Plants

A great deal of the apathy, and sometimes antagonism, that students show toward the study of plants is due to an excessive emphasis on plant morphology and systematics. This section on plants attempts to emphasize the living, functioning plant and utilizes the structure and systematics of plants only as they contribute to the understanding of the basic principles of the plant world.

This will be the student's first contact with multicellular organisms, and here he will see for the first time the specialization of cells in the tissues and organs of a complex multicellular living organism.

The first exercises emphasize the evolutionary development of plants from the mushroom through the seed plants. Great emphasis has been placed on photosynthesis as the process whereby simpler molecules are built into complex carbohydrates and amino acids, essential for animal life on earth. The student will have an opportunity to investigate, in some detail, the phenomenon of photosynthesis, including an investigation of the pigments, the functioning of light, and the structure of the leaf as a photosynthetic organ.

The major structures of plants are then covered, together with such functions as transportation, reproduction, growth, and development.

In the last exercises the students will learn that plants, too, respond to the environment and that they possess hormones which regulate their growth and activity.

Constant stress needs to be placed on the fact that plants are the source of the food necessary for animal life on earth and are the organisms which constantly renew our essential supplies of atmospheric oxygen. On these two bases alone, students can see that a world without plants would be a world without any life as we commonly know it. In addition, the students should recognize that plants illustrate the problems of life in complex multicellular organisms in general; and that plants really lack only well-developed powers of locomotion and coordination to serve as examples of all major living processes in multicellular organisms.

In the *Biology Teachers' Handbook*, you will find a large number of "Invitations to Enquiry" which will aid in class and laboratory discussions of plants and their processes. These invitations include Nos. 3, 4, 6, 7, 11, 14, 19, 27, 29, and 30.

COMPARISON OF PLANTS — SIMPLE OR COMPLEX?

EXERCISE 12-1

TIME: One laboratory period.

MATERIALS: See page 99 in the *Student Laboratory Guide.*

This exercise serves as an introduction to plants. It is to be done while the students are still studying molds and fungi in Chapter 12 of the textbook so that they can progress rapidly in subsequent exercises. Therefore, the students may need help with the ferns, mosses, conifers, and flowering plants.

■ **The purpose of this exercise is to acquaint the student with the great diversity of plants and to notice some of the features which serve to distinguish them from one another. He should attain an understanding of the terms *primitive* and *advanced* in relation to plants.**

SPECIAL INSTRUCTIONS

This exercise introduces the students to a dichotomous type of key without undue emphasis on terminology. As the student has already identified the plants, the key in actuality provides him with characteristics, whereas most keys require prior knowledge of characteristics and then provide names for the plants.

A key-type scoring card is used to call attention to the nature of plant differences and to distinguish relatively primitive from relatively advanced characteristics. Without attempting at this time to develop the theory of organic evolution, the concept of change and resulting diversity may be developed only so far as it is necessary to help explain what is meant by the terms *primitive* and *advanced.*

The use of the score card and the implications of primitive and advanced characters are merely devices to call attention to plant diversity and should not be allowed to distract from the original purpose of the exercise. The numerical values which have been assigned to certain key characters will insure that more advanced plants will receive high scores, and more primitive plants, low scores.

The sources of plant materials have been included in the laboratory guide to get students interested in looking for plant specimens in various areas to use for this exercise. Plenty of material will be available from homes, terraria, supply houses, and greenhouses, even in the middle of winter. A terrarium should have been established long before reaching this unit on plants. We suggest that the terrarium include mosses, liverworts, and some small ferns, all of which can be collected in the fall of the year.

TECHNIQUES

One of the limitations of an exercise such as this is the necessity for keeping it simple. The simpler the dichotomous key, the less widely applicable it will be for specialized materials. Therefore, we caution against the use of marine algae with this score card, as it will not provide the proper classification for such things as *Postelsia, Laminaria, Ulva,* or red algae.

It may be necessary to provide some help with the terminology, although a concerted effort has been made to avoid technical terms in the key. However, such terms as *midrib, parallel veins, netted veins,* or *rhizoids* may need explanation.

We caution against the use of cactus plants in those areas where they are available, as they almost invariably lead to difficulties when handled.

ANSWERS TO QUESTIONS

1. If we assume that evolution of new types from existing forms will occur, we can expect an ever-increasing variety. Most of these will be more complex than their ancestors; some may be less complex.
2. Some of the important differences among plants are whether or not they are grass-green; whether or not they have seeds; whether or not they have roots, stems, and leaves; and whether or not they have flowers.
3. Some of the less important differences are whether the plants are trees or shrubs, are large or small in size, and are aquatic or nonaquatic.
4. Distinguishing important from unimportant differences is based on comparisons of those plants considered primitive and those considered advanced. Characteristics shared by large numbers of species are in general important. Those that distinguish only two very similar species are relatively unimportant.

SUGGESTED READINGS

Books

Benson, L. 1962. *Plant Taxonomy*. Ronald Press, New York.

Lawrence, G. H. M. 1951. *Taxonomy of Vascular Plants*. Macmillan, New York

Swingle, T. B. 1946. A *Textbook of Systematic Botany*. McGraw-Hill, New York

FILMS

Multicellular Plants, Part III: *The Plant Organism*, No. 1, 1961, (sd, c, 24 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Variations on a Theme*, No. 12, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

EXPERIMENTS WITH A SLIME MOLD

EXERCISE

12-2

TIME: Part of one period to start culture, several days for observation and one laboratory period to perform experiment.

MATERIALS: See page 103 in the *Student Laboratory Guide.*

This should accompany the introduction in the textbook to Chapter 12, dealing with the slime mold.

■ **The purpose of the exercise is to observe and to study the reactions to various stimuli of a multinucleate, acellular, cytoplasmic mass – the slime mold, *Physarum polycephalum.***

SPECIAL INSTRUCTIONS

Care must be taken to use only fresh viable cultures. Many cultures marketed by biological supply houses are old and nonviable. Also, care must be taken to insure that the slime mold culture does not dry out.

This exercise requires the preparation of the non-nutrient agar in advance.

Students should be cautioned about using heat. If too much is used, the organism dies.

TECHNIQUES

This exercise lends itself to student teams. The materials listed in the *Student Laboratory Guide* are per team.

At room temperature plasmodia emerge from the sclerotia within a few hours. The plasmodium should be fed daily by sprinkling dry rolled oats (through a coarse sieve) on its surface. Change the water if cloudy and remove the oats not used.

As an alternative to the method of culture given in the *Student Laboratory Guide*, you may use either of the following methods of culture, shown in Figure 12-2-1A and B.

A. Line a Petri dish with two or three layers of filter paper. Saturate this paper with tap water. Near the center, place a pinch of dry oatmeal. Transfer a small piece of the slime mold mycelium to a position beside the oatmeal. If the mold is in the dried form on a small square of filter paper, the square of paper may be placed beside the oatmeal. Dampen the small square with a few drops of water. Add a little water to a larger dish and place the Petri dish in the water in the position shown in the diagram.

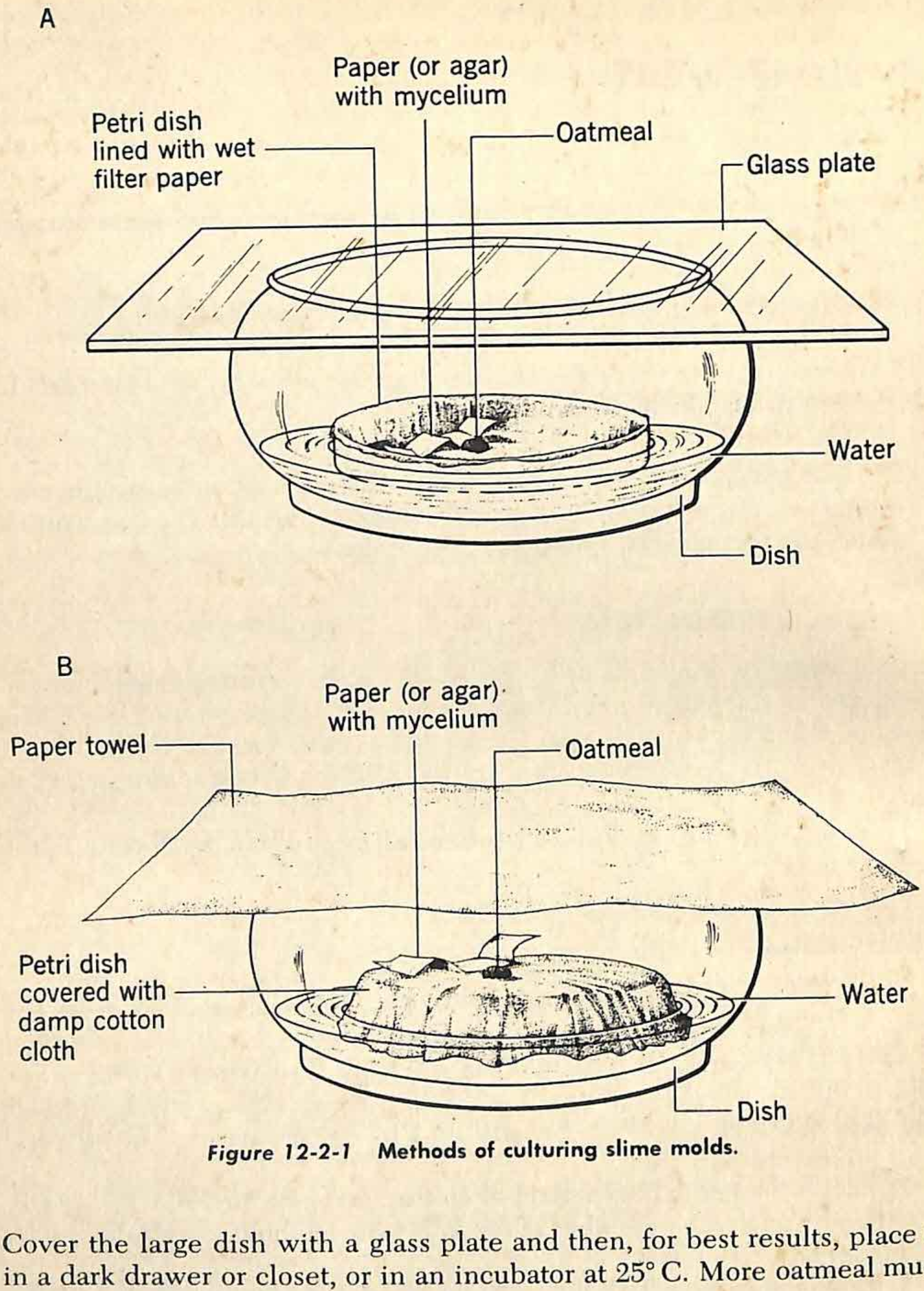

A
Paper (or agar) with mycelium
Petri dish lined with wet filter paper
Oatmeal
Glass plate
Water
Dish
B
Paper (or agar) with mycelium
Paper towel
Oatmeal
Petri dish covered with damp cotton cloth
Water
Dish

Figure 12-2-1 **Methods of culturing slime molds.**

Cover the large dish with a glass plate and then, for best results, place it in a dark drawer or closet, or in an incubator at 25° C. More oatmeal must be added as the mold grows over the paper.

B. Cover a Petri dish with cotton cloth, dampen the cloth, and place the dish and cloth in a larger culture dish containing water about 6 mm deep. Sprinkle a few grains of oatmeal on the top of the cloth. Add the mycelium near the oatmeal. If a number of cultures are to be grown, separate the individual culture dishes by paper toweling, and stack them. Cover the top dish with a glass plate or paper towel.

When the class study is completed, the mold may be preserved in a dried form for use in the following year. Remove the cover of the large culture dish and allow the filter paper with the mold growth to dry for several days. After drying, cut the paper into 1-cm squares, which can then be stored in a sealed envelope. The dormant plant will remain viable for one or two years. In this manner a supply of the mold can be maintained year after year from one original purchase.

Classrooms that have stereoscopic dissecting microscopes will find them more valuable for this study than the compound microscope.

For demonstration, one large class culture can be used if necessary, rather than individual student cultures.

The fruiting bodies can either be found in nature or ordered from a supply house.

The detergent solution is made by adding sufficient household detergent to water to give the solution a slippery feeling.

ANSWERS TO QUESTIONS

1. Students should observe streamers of cytoplasm extending from the initial mass.
2. Cytoplasmic streaming should be seen within the body of the slime mold. This is more frequently observed in the afternoon than in the morning because of the increase in the afternoon temperature.
3. The hole made by the needle is quickly repaired.
4. Pieces will remain independent if the agar has also been cut, thus forming a physical barrier. If only the mold has been cut, the pieces will rejoin.
5. The main body of the mold is an individual.
6. As long as the pieces are separate, they too are individuals. If they join the parent body they are no longer individuals.
7. Gentle warming should increase cytoplasmic streaming; cooling should reduce it.
8. Initially there may be a more rapid streaming of cytoplasm, but the acetic acid will kill the slime mold in the areas it touches.
9. There is no noticeable reaction on the part of the slime mold to the presence of oatmeal. Remember, the organism does not eat the oatmeal but only the bacteria that grow upon the oatmeal.
10. "Fungus" is a correct answer but the student should be in an impasse regarding the plant–animal distinctions at this time. The purpose of this question is to raise doubt as to the validity of "plant–animal" designations at this level.

SUGGESTED READINGS

Books

Alexopoulos, C. J. 1952. *Introductory Mycology.* Wiley, New York

Bessey, E. A. 1951. *Morphology and Taxonomy of the Fungi.* Blakiston (McGraw-Hill), New York

Morholt, E., P. Brandwein, and A. Joseph. 1958. *A Sourcebook for the Biological Sciences.* Harcourt, Brace & World, New York

Articles

Allen, R. D. 1962. "Amoeboid Movement." *Sci. Am.* **206** [2] 112
Bonner, J. T. 1949. "The Social Amoebae." *Sci. Am.* **180** [6] 44
______ 1959. "Differentiation in Social Amoebae." *Sci. Am.* **201** [6] 152

FILMS

Culturing Slime Mold Plasmodium, (sd, c, 6½ min), Thorne
Slime Molds: I. *Life Cycle*, (sd, c, 30 min), Iowa
Slime Molds: II. *Collection, Cultivation and Use*, (sd, c, 19 min), Iowa
Slime Molds: III. *Identification*, (sd, c, 24 min), Iowa

GROWING MUSHROOMS

EXERCISE

12-3

TIME: Thirty-day lead time for mushroom growth plus 3 to 4 days of observation and data recording.

MATERIALS: See page 105 in the *Student Laboratory Guide.*

This exercise should accompany the section on mushrooms that is in Chapter 12 of the textbook.

■ **The purpose of the exercise is to observe the rapid growth of a fungus and to graph the growth rate. In addition, the fruiting bodies of the mushroom will be observed.**

SPECIAL INSTRUCTIONS

Mushroom spawn can be ordered from a biological supply house and planted according to directions. However, biological supply houses also carry inoculated spawn in prepared trays. Although 30 days is given as the time for the mushroom fruiting bodies to appear, they may appear as early as 2 weeks after the receipt of the planted kit. As fruiting body growth is rapid, at least daily observation for signs of growth is necessary.

Breck's (662 Breck Building, Boston 10, Mass.) provides mushroom kits for approximately $4.00, with savings on quantity orders. One kit per laboratory section should be sufficient.

TECHNIQUES

In order to produce fruiting bodies, be sure that the instructions arriving with the kit or spawn are followed to the letter.

In some areas you may find a local supplier of mushrooms from whom appropriate material can be obtained.

The abscissa of the graph for this exercise should be divided into 8-hour intervals, although such intervals will require more frequent periodic observation than use of daily class periods alone would permit. Either the students may be allowed to take fruiting bodies home and measure them at the proper times, or the measurements should be taken by different students throughout the school day. It will be necessary in the latter case to interpolate on the graph between the actual observations of growth.

ANSWERS TO QUESTIONS

1. The membrane ruptures and exposes the gills.
2. The covering membrane protects the gills and the developing spores on the gills from drying out. (Remember this is a land plant.)
3. Mature spores are shed from the gills.
4. The spores are formed on the gills.
5. Formation and dispersal of spores.
6. Hyphae (growing from spores) growing in the compost
7. The stalk grows most rapidly at first, with a commensurate rapid increase in height. As stalk growth slows down, the cap begins a rapid expansion. This relationship should appear on the graph.
8. The stalk elevates the spores on the gills before the spores are ripe for dispersal. As the cap expands, the membrane covering the matured spores ruptures and actual dispersal of the spores occurs.

SUGGESTED READINGS

Books

Christensen, E. C. 1961. *Molds and Men.* University of Minnesota Press, Minneapolis

Kleijn, H. 1962. *Mushrooms and Other Fungi.* Garden City, New York

Articles

Bonner, J. T. 1956. "The Growth of Mushrooms." *Sci. Am.* **194** [5] 97

FILMS

Fungus Plants, 1931, (sd, b&w, 10 min), p or r, EBF

Syngamy and Alternation of Generations in Allomyces—A Water Mold, 1953, (sd, b&w, 20 min), p, Brice

Life of the Molds, 1958, (sd, b&w, or c, 21 min), p or r, McGraw-Hill

Microbiology, Part II: *Fungi,* No. 6, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

GREEN ALGAE— SIMPLE AND COMPLEX

EXERCISE

13-1

TIME: One laboratory period.

MATERIALS: See page 107 in the *Student Laboratory Guide.*

This exercise is to accompany the section on green algae in Chapter 13 of the textbook.

■ **The purpose of this exercise is to introduce the student to the green algae, primitive aquatic organisms, prior to studying evolutionarily more advanced green land plants. An acquaintance with the structure and reproduction of these abundant organisms will contribute to the understanding of plant evolution.**

SPECIAL INSTRUCTIONS

In this exercise the student will see a green alga (*Chlamydomonas*) that he might normally mistake for an animal, a common marine alga (*Ulva*), and a common fresh-water alga (*Oedogonium*).

This exercise is one in a series of exercises dealing with plants from simple to complex. A constant effort must be made to stress the comparative aspects of what the student sees. The green algae, for example, should be compared to the blue-green algae, and subsequently to the higher plants that will be seen later in the laboratory.

The evolutionary theme is an important one in this series of plant exercises and should be kept in mind at all times.

TECHNIQUES

We have concentrated on *Oedogonium*, which is a little more typical of green algae in its method of reproduction than the frequently used *Spirogyra*, which reproduces by conjugation.

Living cultures of *Oedogonium* showing reproductive stages are available at certain times of the year from biological supply houses. *Oedogonium* cultures can be also obtained from Dr. Richard Starr (Department of Botany, University of Indiana, Bloomington, Ind.). If reproductive stages cannot be obtained, good slides showing reproductive stages can be purchased from any biological supply house. There should be one slide for every two students, or two cultures for each class of thirty.

It is not worth the effort to try to culture algae when pure cultures can be obtained at such small cost.

Ulva can be collected fresh at almost any time of the year and can be maintained in a salt-water aquarium for long periods. It also can be ordered living from various biological supply houses.

Pure cultures of *Chlamydomonas* can be purchased from Dr. Richard Starr (address above), as well as from biological supply houses.

When dealing with *Oedogonium*, be sure that the students put only enough material on the slide to be observed clearly but not enough to obscure vision.

ANSWERS TO QUESTIONS

1. The possession of flagella, being motile, and having an eyespot capable of receiving stimuli from light are all animal characteristics.
2. Plantlike characteristics of *Chlamydomonas* are its cell wall, the presence of chlorophyll in a chloroplast, and starch storage.
3. The cells will soon dry up and die, not being adapted for life in the atmosphere.
4. The thallus of *Ulva* is multicellular. It is composed of two cell layers, with only the outer faces of the cells exposed to the environment.
5. *Chlamydomonas* is unicellular. All surfaces of its cell are exposed to the environment.
6. The chloroplast of *Oedogonium* is a network or mesh. It is sometimes called a dishrag chloroplast, because of the large holes in it.
7. The cells form an unbranched filament.
8. It would dry out and die in a short time.
9. The starch is located around little centers of protein called pyrenoids embedded in the chloroplast.
10. An egg.
11. A zygote.
12. The pore allows the sperm to enter the female reproductive structure and unite with its enclosed egg.
13. The cells producing sperms are smaller than those producing eggs.

SUGGESTED READINGS

Books

Smith, G. M. 1950. *The Freshwater Algae of the United States.* McGraw-Hill, New York

Tilden, J. E. 1935. *The Algae and Their Relations.* University of Minnesota Press, Minneapolis

Articles

Milner, H. W. 1953. "Algae as Food." *Sci. Am.* **189** [4] 31

Moore, E. F. 1956. "Artificial Living Plants." *Sci Am.* **195** [4] 118

Weiss, F. J. 1952. "The Useful Algae." *Sci. Am.* **187** [6] 15

FILMS

Microbiology, Part II: *Microorganisms with Chlorophyll*, No. 4, 1961, (sd, c, 32 min), p or r, AIBS(McGraw-Hill)

Reproduction in Plants, 1958, (sd, c or b&w, 13½ min), p or r, Coronet

ALTERNATION OF GENERATIONS

EXERCISE 14-1

TIME: One laboratory period.

MATERIALS: See page 109 in the *Student Laboratory Guide*.

This exercise is to accompany the section on mosses in Chapter 14 of the textbook.

■ **The purpose of this exercise is to acquaint the student with the alternation of generations in green plants and to correlate the alternation of generations with reproduction.**

SPECIAL INSTRUCTIONS

The phenomenon of alternation of generations is discussed in Chapter 13 in the textbook. The organism described there is *Ulva*, where there are two separate plants in the life cycle. When using a moss as an example of alternation of generations, as in this exercise, the student gets a chance to apply the information he has read and to utilize his previous laboratory observations. In a moss, as in *Ulva*, the two plants are still present, but one, the sporophyte, is much reduced and is attached to the gametophyte which is the conspicuous moss plant. The gametophyte is independent. A comparison between *Ulva* and a moss shows an elaboration of the gametophyte and a reduction of the sporophyte in the moss.

Moss plants of the type required can be obtained either living or preserved from biological supply houses.

The theme of evolution is applied to the alternation of generations here in a moss, and later in relation to a fern and a flowering plant. These make interesting comparative examples of plant evolution.

TECHNIQUES

Living moss plants can be collected from the field at any time of the year. If it is winter or a dry season, bring the plants into the laboratory and put them in a terrarium. They will revive and start growing in a day or two.

Living moss plants with mature sporophytes are more difficult to obtain in the field. Preserved specimens can be obtained from a biological supply house.

You will want to show the students how to obtain archegonia and antheridia by squeezing them from the tips of gametophytes. Demonstration slides of archegonia and antheridia can be used, but whole mounts are preferred. The two whole mounts of the filamentous stage (protonema) should be obtained for demonstration from a biological supply house.

Spores of many mosses can be germinated on agar under illumination. This part of the life cycle is not critical to the explanation of the alternation of generations, but it happens to be a part of the moss life cycle and cannot be ignored.

Most moss plants are heterothallic. That is, the male sex organs are on one plant, the female sex organs on another.

ANSWERS TO QUESTIONS

1. Spores.
2. The wind shakes the capsule and the spores are shed. They are then carried or distributed by air currents.
3. Spores have fairly thick walls.
4. The thick wall is composed of waterproof material which keeps the spore from drying out.
5. The leafy shoot of a moss plant has rhizoids which have a function similar to roots and root hairs.
6. Water is required so that the sperm can swim to the archegonium.
7. Reproduction would be impossible in a dry area, so water is required at least during the reproductive (fertilization) stage of the plant.
8. In the base of the archegonium.
9. An embryo.
10. The sporophyte generation.
11. The gametophyte.
12. The sporophyte.
13. In the human life cycle there is no stage corresponding to the vegetative gametophyte.

SUGGESTED READINGS

Books

Bold, H. C. 1960. *The Plant Kingdom*. Prentice-Hall, Englewood Cliffs, N.J.
Smith, G. M. 1955. *Cryptogamic Botany*. Vol. 2. McGraw-Hill, New York

FILMS

Multicellular Plants, Part III: *Variations on a Theme*, No. 12, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Contains material on alternation of generations and sexual and asexual reproduction.

Miracle of Moss, 1945, (sd, b&w, 10 min), p or r, Almanac

S.C.E.R.T., West Bengal
Library
Calcutta
25/3, B. C. Road

A PRIMITIVE VASCULAR PLANT

EXERCISE 14-2

TIME: One laboratory period.

MATERIALS: See page 111 in the *Student Laboratory Guide.*

This exercise is to accompany the section on vascular plants in Chapter 14 of the textbook.

■ **The purpose of this exercise is to acquaint the students with characteristics of a primitive vascular plant and its mode of reproduction.**

SPECIAL INSTRUCTIONS

This exercise takes the student along the road of plant evolution to the appearance of vascular tissue in the sporophyte and the elaboration of the sporophyte in the alternation of generations.

The species of fern chosen for study in the laboratory will vary with the availability of fresh greenhouse material or the abundance of material in the natural environment. Plants of Boston fern (*Nephrolepsis*) or Christmas fern (*Polystichum*), either potted or growing in nature, are available in many localities. One or more living fern plants freshly removed from the soil so as to expose the underground stems (rhizomes) and roots should be available for study. After the laboratory study is completed these plants may be repotted for future use.

TECHNIQUES

A spore-bearing leaf (fertile leaf) and a nonspore-bearing leaf (sterile leaf) should be furnished for each four students. Fresh specimens may be used to demonstrate the wide variation in leaf types in ferns. Simple entire leaves, lobed leaves once-pinnate, and twice-pinnate leaves may be shown if material is available. Kodachromes of tropical tree ferns may also be used to illustrate further the variety of fern types.

In order to demonstrate the nature and behavior of fern sporangia, care must be exercised in selecting leaves at the right stage of maturity to show the ejection of spores. This calls for testing the various leaves before the laboratory period begins. Keep the leaves in a moist plastic bag or vasculum until the time of the study. The heat of a bright light held close to the leaves should then cause drying out and ejection of spores. Sporangial dehiscence may also be demonstrated by placing intact sporangia in a drop of glycerol and examining them under the low power of the microscope.

Fern gametophytes may be found growing on moist soil beneath mature sporophytes in the greenhouse or in their natural habitats.

If living fern gametophytes are not available, prepared slides, one for each two students, can be purchased from biological supply houses.

Demonstrations of the cross section of fern rhizomes can be obtained from a biological supply house. Only two are necessary for demonstration purposes.

ANSWERS TO QUESTIONS

1. The ability to conduct materials rapidly and the additional aerial support that permits leaves to be spread in the light and spores to be widely dispersed, both of which, possible because of the vascular tissue, contribute to the success of the plants.
2. At the tip of the prostrate stem.
3. Coiled, and sometimes known as fiddleheads or croziers.
4. It is more complex, longer-lived, conspicuous, and independent at maturity. The moss sporophyte is less complex, smaller, of short duration, and dependent on the gametophyte for its food.
5. The spore capsule.
6. No, because they are small and easily overlooked.
7. The fern gametophyte is shorter-lived than the moss gametophyte and the moss gametophyte is relatively more conspicuous than that of a fern, because it is erect. Both are autotrophic.
8. Rhizoids.
9. Moss.
10. Motile fern sperms can be seen swimming in the water.
11. Water is essential in order for the sperms to swim to the egg.
12. It is similar. Ferns, algae, and mosses all require water for fertilization.
13. Zygote.
14. The sporophyte.
15. The sporophyte.
16. Both generations.
17. Because they require free water for fertilization. This restricts them to moist, humid environments.

SUGGESTED READINGS

Books

Foster, A. F., and E. M. Gifford, Jr. 1959. *Comparative Morphology of Vascular Plants.* W. H. Freeman, San Francisco

Smith, G. M. 1955. *Cryptogamic Botany.* Vol. 2. McGraw-Hill, New York

FILMS

Life Story of a Fern, 1953, (sd, b&w, 16 min), p or r, UW

Multicellular Plants, Part III: *Stems,* No. 7, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Covers vascular tissues.

Multicellular Plants, Part III: *Variations on a Theme*, No. 12, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Highlights the life cycle and the alternation of generations of the fern.

THE IMPORTANCE OF SEEDS

EXERCISE 14-3

TIME: One laboratory period.

MATERIALS: See page 113 in the *Student Laboratory Guide.*

This exercise is to accompany the discussion of vascular plants in Chapter 14 of the textbook.

■ **The purpose of this exercise is to continue the study of the evolution of flowering plants. The gymnosperm, as represented by the pine, gives the student the opportunity to observe the structure of an advanced vascular plant and to study its reproductive cycle for comparative purposes.**

SPECIAL INSTRUCTIONS

Continue to emphasize the evolutionary nature of this sequence of laboratory exercises and emphasize that the conifer, as exemplified by the pine, is used as an example of a seed-producing plant. The emphasis should be placed on the importance of the seed as a survival mechanism. It is an important step in the evolution of plants, especially when we remember that two-thirds of all plant species form seeds.

TECHNIQUES

Fresh pine branches can be collected at any time of the year. Only in the spring, however, will the branches have both pollen and seed cones.

Old seed cones are available almost any time of the year. Preserved young seed cones and pollen can be obtained from biological supply houses.

One pollen cone for each student, one old seed cone for four students, and one pine seed for each student will be sufficient.

ANSWERS TO QUESTIONS

1. Variable according to the species of pine used.
2. Spiral.
3. It is possible to see the terminal bud scars for succeeding years; counting the number of rings of terminal bud scars will give the age of the twig.
4. The pine sporophyte is a tree or a shrub and is larger than most ferns. Both are photosynthetic.
5. It is advantageous to have large numbers of pollen cones because the more pollen produced, the wider its dispersal by the wind and the greater the chance of pollination and fertilization.
6. Pollen grains.
7. They form pollen tubes and sperm cells that fertilize the eggs.
8. By the surrounding tough cone scales.
9. Through the pollen tube.
10. As the cone scales open, the seeds fall.
11. By the embryo for growth.
12. The pine seed, with its thick coat, abundant stored food, and embryo, has a much better chance of survival than the tiny spore of either the moss or the fern.

SUGGESTED READINGS

Books

Bold, H. C. 1960. *The Plant Kingdom.* Prentice-Hall, Englewood Cliffs, N.J.

Articles

Koller, D. 1959. "Germination." *Sci. Am.* **200** [4] 75
Salisbury, F. B. 1957. "Plant Growth Substances." *Sci. Am.* **196** [4] 125
Went, F. W. 1955. "The Ecology of Desert Plants." *Sci. Am.* **192** [4] 68

FILMS

Multicellular Plants, Part III: *Seeds and Germination*, No. 2, 1961, (sd, c, 24 min), p or r, AIBS(McGraw-Hill)
Seed Dispersal, 1951, (sd, b&w, 14 min), p or r, UW
Seed Dispersal, 1956, (sd, b&w or c, 11 min), p or r, EBF
Growth of Seeds, 1958, (sd, b&w or c, 14 min), p or r, EBF
Gymnosperms, 1962, (sd, b&w or c, 16 min), p or r, EBF

THE PIGMENTS IN A LEAF

EXERCISE
15-1

TIME: One laboratory period.

MATERIALS: See page 115 in the *Student Laboratory Guide.*

This exercise is to accompany Chapter 15, on photosynthesis.

■ **The purpose of the exercise is to allow the students to master the technique of pigment extraction and to determine what pigments occur in green leaves.**

SPECIAL INSTRUCTIONS

This exercise will show the student that there is more than one kind of pigment present in green leaves. This is probably best done as a demonstration. However, there is no reason why the students, with some supervision, cannot make their own extractions and separations.

Some words of warning are required in this and other similar exercises. Ethyl alcohol will be used in several exercises as a solvent for leaf pigments. The alcohol must be boiling to do the job properly. *Never heat alcohol over a flame of any kind.* Use instead an electric hot plate or a water bath in good working order. Have a CO_2 fire extinguisher handy to smother a fire if one should get started.

If students are involved in making the separation of yellow and green pigments, they should be warned not to inhale the carbon tetrachloride fumes, which are poisonous, and also not to get any on the skin.

TECHNIQUES

Do not cover the boiling alcohol with a glass plate. Caution the students about using boiling fluids which will be dangerous if spilled.

ANSWERS TO QUESTIONS

1. No.
2. Green pigments are not water-soluble.
3. Green pigments are soluble in ethyl alcohol.
4. Light green.
5. The upper layer is yellow or yellow-green, the lower layer is dark green.
6. There are at least two kinds of pigments present.
7. The green ones.
8. The yellow ones.

SUGGESTED READINGS

Articles

Frank, S. 1956. "Carotenoids." *Sci. Am.* **194** [1] 80

Rabinowitch, E. I. 1953. "Progress in Photosynthesis." *Sci. Am.* **189** [5] 80

Thimann, K. V. 1950. "Autumn Colors." *Sci. Am.* **183** [4] 40

White, P. R. 1950. "Plant Tissue Cultures." *Sci. Am.* **182** [3] 48

FILMS

Microbiology, Part II: *Microorganisms with Chlorophyll*, No. 4, 1961, (sd, c, 32 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Chlorophyll*, No. 4, 1961, (sd, c, 26 min), p or r, AIBS (McGraw-Hill)

CHLOROPHYLL AND PHOTOSYNTHESIS

EXERCISE

15-2

TIME: Part of one laboratory period.

MATERIALS: See page 117 in the *Student Laboratory Guide*.

This exercise is to accompany Chapter 15 on photosynthesis.

■ **The purpose of this exercise is to determine whether chlorophyll in the leaf is necessary for photosynthesis or whether nonpigmented portions of the leaf can carry on photosynthesis.**

SPECIAL INSTRUCTIONS

In this simple experiment it will be necessary to explain to the students that starch, for which we can easily test, is the first readily detectable product of photosynthesis. Therefore, if starch is present, photosynthesis must be occurring at those places. If starch is not present, photosynthesis has not occurred there.

This exercise may emphasize the nature of scientific inquiry by having the student form a hypothesis that chlorophyll is or is not necessary for photosynthesis and then, by utilizing the results of this exercise, arrive at some conclusion regarding his original hypothesis.

"Invitations to Enquiry," No. 19, "Photosynthesis," in the *Biology Teachers' Handbook* introduces the topic of serial causation and may be used at this point.

ECHNIQUES

This exercise makes a simple and easy demonstration, but it is equally imple for the students to do. How it is used should depend on the avail-bility of time and equipment.

You should get plants with green and white variegated leaves, not red nd green. Geranium and *Coleus* are best to use and can be obtained at a ocal greenhouse regardless of the time of the year when the exercise is to e done.

Students should be cautioned, if they heat the alcohol mixture with a hot plate, that boiling alcohol is dangerous and highly inflammable.

ANSWERS TO QUESTIONS

1. The two patterns are the same, indicating that the starch was formed only in those parts of the leaf where chlorophyll was present.
2. Sugar is a soluble substance and is very difficult to demonstrate while still in the leaf. Because starch is insoluble and its presence is easily demonstrated with iodine solution, we test for it instead.
3. If starch is the first readily detectable product of photosynthesis, then chlorophyll is obviously a factor in its formation.

SUGGESTED READINGS

Books

Calvin, M., and J. A. Bassham. 1962. *The Photosynthesis of Carbon Compounds.* W. A. Benjamin, New York

Also see references for Exercise 15-1.

FILMS

See those listed for Exercise 15-1.

LIGHT IN CARBOHYDRATE SYNTHESIS

EXERCISE
15-3

TIME: One laboratory period.

MATERIALS: See page 119 in the *Student Laboratory Guide.*

This exercise is to accompany the section on light and photosynthesis in Chapter 15 of the textbook.

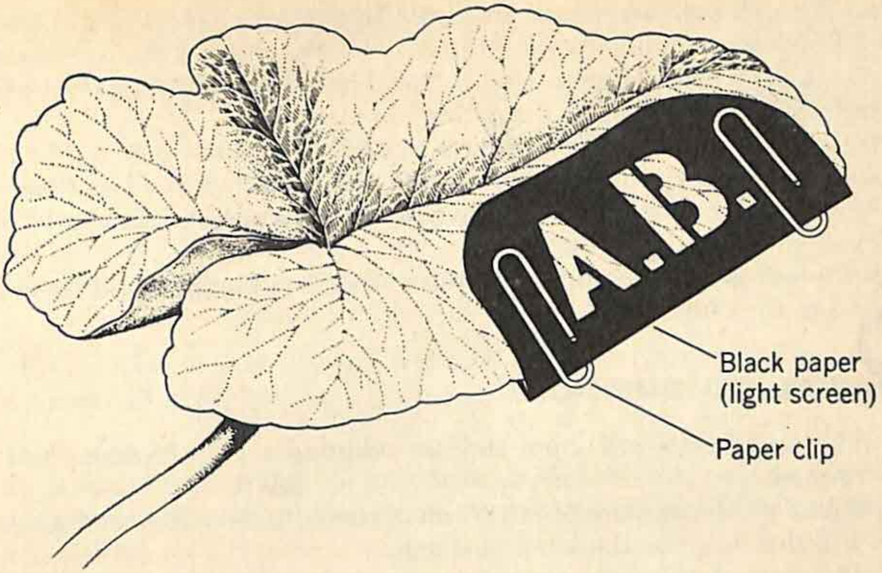


Figure 15-3-1 **Geranium leaf with light screen attached.**

■ The purpose of this exercise is to observe the effect of light on photosynthesis in green plants by using formation of starch as a photosynthetic indicator.

SPECIAL INSTRUCTIONS

If you have not already performed Exercise 15-1, note that the technique for chlorophyll extraction, which is not repeated for this exercise, is introduced there. You may wish to refer the students who undertake this exercise to Exercise 15-1. Be sure that you have reviewed the precautions outlined in 15-1 before performing this exercise.

The topic of linear relation is introduced through the subject of light intensity and photosynthesis in "Invitations to Enquiry," No. 27 in the *Biology Teachers' Handbook* and may be used here.

TECHNIQUES

Preparation for this exercise must begin at least 4 days before the class period in which the exercise is used. Preparation is started by depleting starch in several geranium plants by placing them in a dark room for 3 days. At some time during this interval, prepare the light screens of black construction paper. The screen must cover the lower as well as the upper surface of the leaf. Cut some design in the screen. This will be reproduced on the leaf when the student tests the leaf for starch distribution. A good contrasting photographic negative will work nicely if the lower surface of the leaf is backed with black paper so that the only light reaching the leaf passes through the negative placed over the upper surface of the leaf.

Make enough light screens so that each student can have a leaf to test.

Just before removing the plants from the dark room put the light screens in place, as shown in Figure 15-3-1. Now bring the plants out into the light for at least 12 hours before using them in class.

For morning classes you will have to take the plants out of the dark and place them under artificial light during the night before class time. For afternoon classes the plants will have to be taken from the dark and placed under light in the morning.

A student group can do this exercise as a special project and report the results to the class.

ANSWERS TO QUESTIONS

1. Photosynthesis will stop and no additional carbohydrate will be formed.
2. It will use foods stored in the form of starch, and the leaves of the plant will thus become depleted of starch.
3. The part of the leaf covered by the light screen remains colorless, indicating lack of starch formation. It could be concluded that photosynthesis did not occur in the absence of light in any part of the leaf from which light was excluded, despite the fact that the rest of the leaf was illuminated.

SUGGESTED READINGS

Books

Galston, A. W. 1961. *Life of the Green Plants.* Prentice-Hall, Englewood Cliffs, N.J.
Steward, F. C. (ed.) 1960. *Plant Physiology.* Vol. 1B. Academic Press, New York

Articles

Arnon, D. I. 1960. "The Role of Light in Photosynthesis." *Sci. Am.* **203** [5] 104
Rabinowitch, E. I. 1948. "Photosynthesis." *Sci. Am.* **179** [2] 24
—— 1953. "Progress in Photosynthesis." *Sci. Am.* **189** [5] 80

FILMS

Multicellular Plants, Part III: *Chlorophyll*, No. 4, 1961, (sd, c, 26 min), p or r, AIBS (McGraw-Hill)

Multicellular Plants, Part III: *Role of the Green Plant*, No. 5, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)

The Riddle of Photosynthesis, 1955, (sd, b&w, 12½ min), p or r, Handel

Leaves, (sd, b&w, 11 min), p or r, EBF

Carbon Fourteen, 1954, (sd, b&w, 12 min), p or r, EBF

THE STOMA—GATEWAY INTO A LEAF

EXERCISE

15-4

TIME: One laboratory period.

MATERIALS: See page 121 in the *Student Laboratory Guide*.

This exercise is to accompany the section on the activities of the stomata in Chapter 15 of the textbook.

■ **The purpose of this exercise is to uncover for the student the role the stomata play in relation to absorption of carbon dioxide and photosynthesis.**

SPECIAL INSTRUCTIONS

The operation of the stomata in relation to photosynthetic activity of the leaf affords an excellent example of regulation. This exercise, which elaborates on the textual material dealing with stomata, provides a fine opportunity to discuss the theme of regulation (homeostasis).

In such a discussion there is ample opportunity to review the processes of diffusion, turgor pressure, and the role of carbon dioxide in photosynthesis.

TECHNIQUES

The thin, tender leaves of plants such as snapdragons are best for removal of the lower epidermis. However, leaves of many plants can be used to obtain a view of stomata. The Shasta daisy, iris, and daffodil are a few that can be used with considerable success. One or two plants may be sufficient for the entire class.

Do not use wilted leaves. Keep the plants in water or in pots. Pick the leaves as they are required. Keep the plants in a lighted room but not in direct sunlight.

ANSWERS TO QUESTIONS

1. There are no chloroplasts in the epidermal cells surrounding the guard cells.
2. Chloroplasts are present in the guard cells.
3. The inner walls of the guard cell are thicker than the outer ones.
4. The important gases which will be involved are water vapor, oxygen, and carbon dioxide. Of course all other gases will be free to diffuse in

or out of the leaf, but they will not contribute to photosynthesis.

5. Carbon dioxide is the important gas for photosynthesis.
6. About 0.03 to 0.04%.
7. Depending upon environmental conditions, both water vapor and oxygen may diffuse out through the stomata.
8. Yes, by the activity of the guard cells.
9. In a healthy plant which has not been in bright sunlight, the stomata are open.
10. The shape of the guard cells will change so that there will be no open stomata.
11. The salt solution has caused the guard cells to lose water and thus to close the stomata.
12. Guard cells contain chloroplasts, while epidermal cells lack these plastids. The opening and closing of the guard cells is regulated in part by the amount of carbohydrates synthesized. This in turn determines the turgidity of the guard cells.

SUGGESTED READINGS

Books

Heath, O. V. S. 1959. "The Water Relations of Stomatal Cells and the Mechanisms of Stomatal Movement." *Plant Physiology.* Vol. 2, Academic Press, New York

FILMS

Multicellular Plants, Part III: *Role of the Green Plant*, No. 5, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)

Presents functions of the stoma in photosynthesis and respiration by means of a leaf peel.

THE LEAF— A PHOTOSYNTHETIC ORGAN

EXERCISE

15-5

TIME: One laboratory period.

MATERIALS: See page 123 in the *Student Laboratory Guide.*

This exercise is to accompany the section on the leaf in Chapter 15 of the textbook.

■ **The purpose of this exercise is to correlate the structure of the leaf with its function as a photosynthetic organ.**

SPECIAL INSTRUCTIONS

This exercise offers a fine opportunity to emphasize one of the themes of the BSCS program, namely, the complementarity of structure and function. In this exercise the student will see a superbly structured organ, the leaf, ideally suited for the function of photosynthesis. Every effort should be made to correlate the structures and the functions of all the parts.

The object of this exercise is to consider the structure of the leaf from the standpoint of its primary function, photosynthesis. Do not bring in a multitude of leaf types, as this will only distract from the main point to be emphasized.

The primary purpose of the drawing (Figure 15-5-1) in the *Student Laboratory Guide* is for the student to locate the chloroplasts properly and to help him interpret the slide. No labels are asked for, since the primary object is to relate the structure of the leaf to the functions involved in photosynthesis.

"Invitations to Enquiry," No. 29 in the *Biology Teachers' Handbook*, covers nonlinear polynomial of degree greater than 1 through the subject of growth regulation in the leaf.

TECHNIQUES

If you wish to keep the *Student Laboratory Guide* intact, the student can copy Figure 15-5-1 from the guide or it can be supplied to him separately on a hectographed or otherwise duplicated sheet.

One plant with numerous leaves can often supply the entire class.

Slides of sectioned lilac or privet leaves are available from most biological supply houses and these show typical leaf structure.

If you wish to make freehand cross sections of living material, the elder pith technique is excellent.

Take a piece of elder pith about 5 cm long and cut it lengthwise down the middle (Figure 15-5-1). With this, make a vise in which a piece of leaf can be held between your thumb and forefinger while you make the section. Put the leaf fragment between the elder pith halves so that when

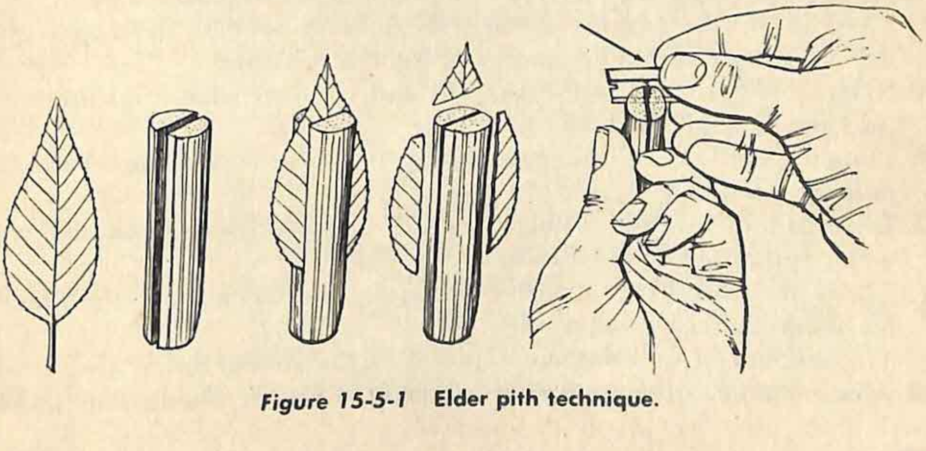


Figure 15-5-1 **Elder pith technique.**

thin slices of pith are cut, thin sections of the leaf are also made. Wet the surface of the pith; with the razor blade slice thin sections across the top of the elder pith clamp containing the plant specimen. The razor blade should be pulled toward you by drawing the blade obliquely across the pith. When the cut is completed, the section of leaf should be placed in a watch glass containing water. Several sections should be made until one that is really thin is obtained. This one should be put on a slide in a drop of water, covered with a *clean* cover glass, and observed under the microscope.

Elder pith, recommended for making freehand sections, can be obtained from biological supply houses (Cambosco Sci. Co., Boston, 35). It comes in bundles. At least two bundles per class are recommended.

Making freehand sections using elder pith is a rapid technique that makes working with living plant materials a lot more fun for the student. It is a good idea for you to practice making sections before the class period so that you can demonstrate how it is done. This technique may be used in later exercises. As in any exercise in which the razor blade is used, care must be taken that the students do not cut themselves. Even though the instructions call for pulling the blade toward the student, this should not result in difficulty if it is done slowly and with care.

If students prepare cross sections they might also try staining them with methylene blue or some other stain.

ANSWERS TO QUESTIONS

1. It exposes more surface to light energy, thus increasing the total amount of photosynthesis that can be accomplished by the leaf. This in no way, however, affects the efficiency of photosynthesis.
2. In spirals, whorls, alternately, or oppositely.
3. The various arrangements reduce the amount of shading of the lower leaves by the upper leaves of the plant and allow some light to penetrate to the lowest leaves.
4. They are usually disk-shaped structures.
5. The spongy and palisade cells.
6. Because they contain the largest number of chloroplasts per cell.
7. They are on the insides of the cells directly beneath the upper epidermis, or as close to the source of light as possible.
8. Xylem cells provide some support and conduct water and minerals into and through the leaf.
9. Phloem conducts soluble foods away from the leaves into the other parts of the plant.
10. It keeps the leaf from drying out, thus insuring that there is plenty of water available for photosynthesis.
11. There are many large intercellular spaces between the cells. Thus a lot of cell surface is exposed.
12. The amount of cell surface exposed in the intercellular spaces into which stomata open provides a large area for the absorption of CO_2.

13. It goes into solution on the wet surfaces of cells bordering the intercellular spaces.

SUGGESTED READINGS

Books

Galston, A. W. 1961. *The Life of the Green Plant.* Prentice-Hall, Englewood Cliffs, N.J.

Articles

Ashby, E. 1959. "Leaf Shape." *Sci. Am.* **181** [4] 22

Jacobs, W. P. 1955. "What Makes Leaves Fall." *Sci. Am.* **193** [5] 82

FILMS

Multicellular Plants, Part III: *Leaves,* No. 3, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Leaves, (sd, b&w, 11 min) p or r, EBF

The Riddle of Photosynthesis, 1955, (sd, b&w, 12½ min) p or r, Handel

THE STEM—STRUCTURE AND FUNCTION

EXERCISE 16-1

TIME: One laboratory period.

MATERIALS: See page 125 in the *Student Laboratory Guide.*

This exercise is to accompany the portion on the stem in Chapter 16 of the textbook.

■ **The purpose of this exercise is to determine the relationship between the structure and the function of the various parts of a plant stem.**

SPECIAL INSTRUCTIONS

The major purpose of this exercise should not be obscured by excessive detailed considerations of structure. The critical issue is the relationship of structure to function in the plant stem. This is an excellent exercise with which to reinforce the theme of the complementarity of structure and function.

The bean seeds for this exercise should have been planted two or three weeks before they are to be used. Each student should have his own plant. The student will make a diagram of the general regions of the stem, coloring in the various regions with colored pencils. Have the student key the colors—that is, red for supporting tissue, blue for xylem, brown for phloem, yellow for waterproof layers, and green for storage tissue. Obviously, other colors as available can be substituted.

TECHNIQUES

The student can draw a good circle by using a Petri dish or a jar lid if compasses are not available.

If new stem slides must be ordered, those of sunflower, bean, or buttercup are excellent for this purpose. The slides should be of young stems and should show little or no cambial activity.

The idea of using colored pencils to fill in various parts of the diagram is optional, but it makes it easier to check the student's accuracy of observation. Labels can be used if desired.

ANSWERS TO QUESTIONS

1. No. The longer internode is the lowest. The internodes higher on the plant are successively shorter.
2. Green.
3. It is photosynthetic.
4. No. When woody stems (tree trunks) mature, they lose their photosynthetic ability.
5. One cell thick.
6. In the vascular (conducting) bundles.
7. As they have no cytoplasm, they are dead.
8. They support as well as conduct.
9. They should have been colored red previously, the addition of blue will produce a purple color for xylem.
10. In the vascular bundle, just to the outside of the xylem.
11. The outside surface of the epidermis.
12. The large cells of the cortex.

SUGGESTED READINGS

Books

Foster, A. S. and E. M. Gifford. 1959. *Comparative Morphology of Vascular Plants.* W. H. Freeman, San Francisco

Articles

Biddulph, S. and O. Biddulph. 1959. "The Circulatory System of Plants." *Sci. Am.* **200** [2] 44

Esau, K. 1950. "Development and Structure of the Phloem Tissue." Part 2, *Bot. Rev.* **16** 67–114

______ 1954. "Primary Vascular Differentiation in Plants." *Biol. Rev.* **29** 46-86

Salisbury, F. B. 1957. "Plant Growth Substances." *Sci. Am.* **196** [4] 125

Williams, S. 1953. "Wood Structure." *Sci. Am.* **188** [1] 64

FILMS

Multicellular Plants, Part III: *Stems*, No. 7, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

THE ROOT—AN AID TO PLANT NUTRITION

EXERCISE
16-2

TIME: One laboratory period.

MATERIALS: See page 127 in the *Student Laboratory Guide.*

This exercise is to accompany the section on roots in Chapter 16 of the textbook.

■ **The purpose of this exercise is to correlate the structure and function of the root. Special emphasis is placed on the role of the root in nutrition.**

SPECIAL INSTRUCTIONS

The grass seedlings for this exercise should be started about 10 days before the exercise is to be done. If radish seedlings are used, they should be started about 6 days before their required use in class.

This exercise concerns itself with complementarity of structure and function in relation to the root and plant nutrition. This is not so much an exercise in root morphology as it is an exercise in the role of the root in the life of the plant.

"Invitations to Enquiry," Nos. 6 and 7 in the *Biology Teachers' Handbook* deal with planning and control of experiment, through plant nutrition, and may be used in conjunction with this exercise.

TECHNIQUES

The materials listed in the *Student Laboratory Guide* are for the entire class.

For the germination of radish seeds, cut a disk of dark-colored blotting paper to fit in the bottom of a Petri dish. Put about six radish seeds, evenly spaced, on the filter paper and water each dish so that the paper is wet. Place the covers on. After the young roots have appeared, do not pour water on them and do not leave the covers off the Petri dishes, as this will ruin the root hairs.

The most satisfactory grass for germination is red-top grass. It can be obtained from any large seed store. As all grass is a little temperamental about germination, it may be well to start two or three batches at different times. One batch should then be ready for class use. It is recommended that you start the first batch two weeks in advance of class, the next 10 days, and the last, a week ahead of time.

In each case, fill a finger bowl full of water and sprinkle the grass seed on the surface. It will float. The roots and root hairs formed will not be disturbed when they are ready for use by the students. From past experience we know that your students will look at the young shoot and not the root. A word of caution from you will save a lot of time at this point. The students must treat the seedlings with care when they put them on the slide. The root hairs are delicate and are easily twisted and broken. Remember to have the students use forceps to pick out the seedlings from the finger bowl.

The grass plant chosen should have as full a root system as possible.

Whole carrots, with their tops on, are best for the exercise, but they are hard to get in the average grocery store.

The preferred root cross section is that of a buttercup. Other root slides can be used if already available.

ANSWERS TO QUESTIONS

1. The carrot has a single fleshy tap root. The grass root system is fibrous, composed of many small, slender roots.
2. On the older part of the root, farther from the tip.
3. The root hairs would be broken off in the soil.
4. Unicellular.
5. Extensions of individual cells in the upper epidermis of the root.
6. Living.
7. The functions are opposite. The epidermis of stem and leaf is cutinized and holds moisture in the plant. The root lacks this cutinized layer, as its function is absorption of water.
8. Diffusion.
9. The xylem of the root forms a solid central strand and is not divided into many strands as in the stem.
10. The phloem is located between the radiating arms of xylem.
11. Phloem conducts soluble food from the leaves to the root.
12. The cortex.
13. The chief difference is in the distribution of the supporting tissues.

SUGGESTED READINGS

Books

Esau, K. 1953. *Plant Anatomy.* Wiley, New York

Articles

Salisbury, F. B. 1957. "Plant Growth Substances." *Sci. Am.* **196** [4] 125

FILMS

Multicellular Plants, Part III: *Roots*, No. 6, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Root Development, 1951, (sd, b&w, 10 min), p or r, UW

Roots of Plants, 1957, (sd, b&w or c, 10 min), p or r, EBF

TRANSPIRATION IN PLANTS

EXERCISE

16-3

TIME: One laboratory period for all parts, with an additional 5 days of observations for Part B.

MATERIALS: See page 129 in the *Student Laboratory Guide.*

This exercise is to accompany the section on ascent of water and dissolved minerals in plants in Chapter 16 of the textbook.

■ **Each part of this exercise has a specific purpose. The purpose of Part A is to show that water vapor is lost from leaves. Part B shows how much water is lost from the stem and leaves of a living plant in a given period of time. In Part C the force developed by transpirational pull is demonstrated.**

SPECIAL INSTRUCTIONS

The class may be divided into teams to perform the three parts of this experiment; or alternatively, the exercise may be done in whole or in part as a demonstration.

The demonstration of transpirational pull should be started at the beginning of the class. If the apparatus is working properly it will take thirty to forty-five minutes for the completion of Part C.

Part B can be set up during one class period. However, 5 subsequent days of observations and weighing are necessary in order to reach the conclusions called for.

It is recommended that Part A be done by all members of the class. In that case, sufficient geranium plants and cobalt chloride paper for everyone will be needed.

TECHNIQUES

Part A: Water Loss from Leaves

The leaves are left on the plant so they can be used over and over again. The cobalt chloride paper can be made ahead of time by soaking strips of filter paper in 3% cobalt chloride solution. The water solution is red and so is the paper when it is wet. As the strips dry, they will turn blue. Store the strips in a dry container or a calcium chloride dessicator.

Part B: Measuring the Volume of Water Lost

Rather sturdy scales (Harvard type) are needed to measure the amount of water loss. Remember you are weighing a whole plant in a pot with soil.

Check the plastic bags used in Part B to make sure they have no holes in them. When you cover the pots with the bags make sure the bags are tied snugly to prevent water loss.

Part C: The Force of Transpirational Pull

This part can also be a demonstration. Although its preparation may seem a little complicated, it is worth the effort because it provides a very graphic demonstration of the great force of transpirational pull and the cohesive force of water molecules. Following is a detailed account of the materials needed and the procedure for assembling the potometer. (See Figure 16-3-1.)

Two ring stands with supporting rods approximately 1-m long
Ten utility clamps with extensions
Two 1-m lengths of 0.25-mm bore capillary tubing
Three finger bowls
Mercury
Two watch glasses
Two 125-ml wide-mouthed bottles
Two 2-holed No. 8 rubber stoppers
One 16-cm length of glass tubing
One 64-cm length of rubber tubing (5-mm diameter)
Glass mouthpieces (one per student)
One plant branch (preferably pine)
70% ethyl alcohol
Two meter sticks
Sharp knife
One liter of boiled water

Preparing the Apparatus

Both 1-m pieces of capillary tubing must be bent into a U at one end. Also bend the 16-cm glass tubing into a U. It should be inserted into one of the holes of a 2-holed rubber stopper.

Before inserting the living branch, set up the rest of the apparatus, using Figure 16-3-1 as your guide. You will have to make adjustments in the clamps to align the bottle and stopper.

Boil at least a liter of water for 5 minutes.

When ready for the branch, remember that white pine branches work best. In any event, a branch of some kind of pine is to be preferred because it transpires more rapidly than the branches of broad-leaved trees. Preparing the pine branch is one of the most critical steps in the experiment. To collect branches you need sharp pruning shears and a small bucket of water. Three or four branches should be collected no more than a day before they are to be used.

Keep in mind that the end of the branch is going to be pushed through one hole of the rubber stopper and must fit very tightly. Immediately after you cut the branch, place the cut end into the water in the bucket.

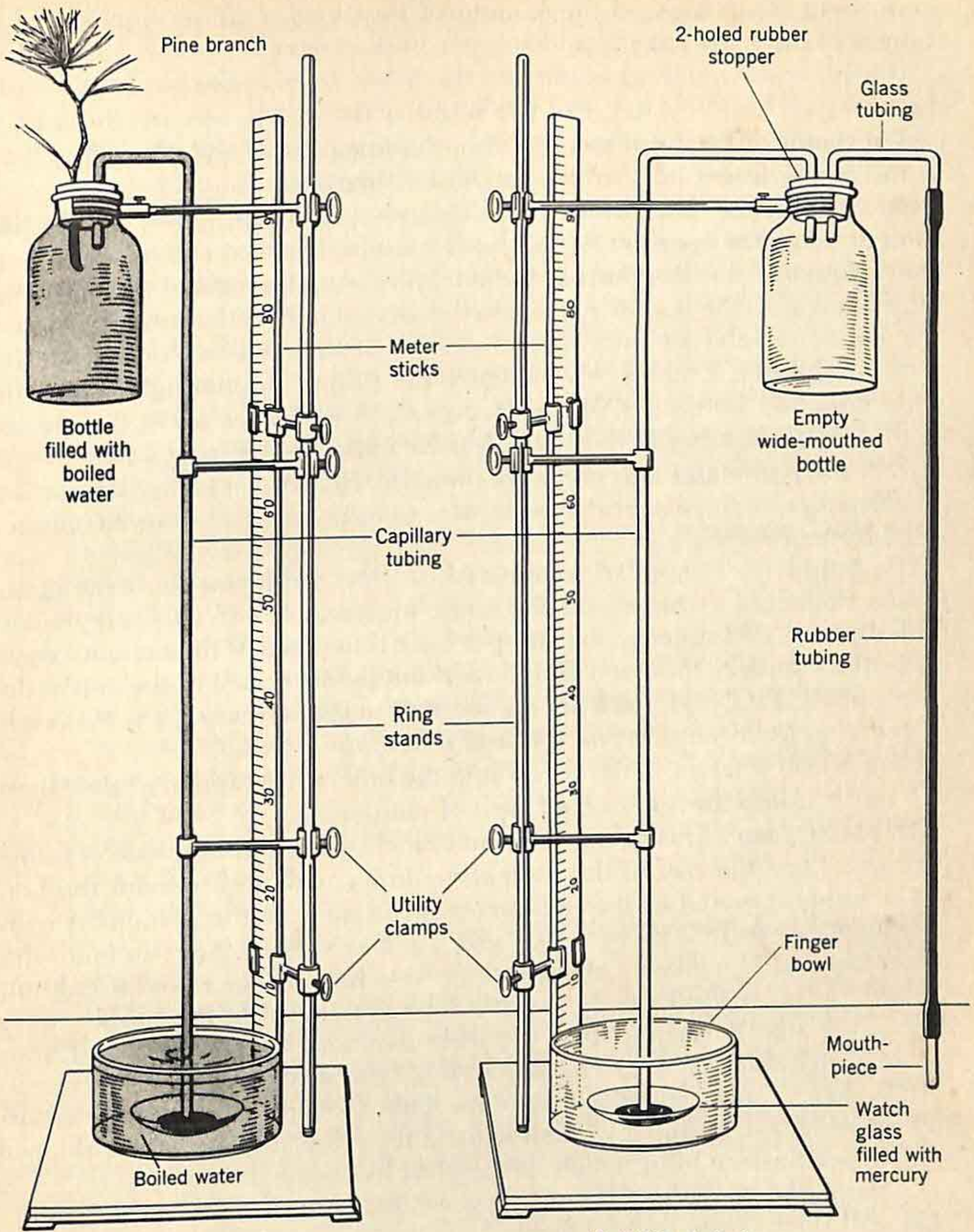


Figure 16-3-1 **Potometer apparatus.**

While it is still submerged in the bucket, trim about 3 cm of bark from the cut end of the branch. Still under water, work the trimmed end through the hole in the top of a rubber stopper. When the stopper reaches the bark, continue to push the branch through, but try not to peel the bark back; work it down into the hole. The trimmed end of the branch should finally extend about 3 cm beyond the bottom of the stopper. This should give a *tight fit*! Leave the branch and stopper in the water.

Fill the wide-mouthed bottle and the finger bowl of the potometer with the now cool boiled water. Fill two watch glasses with mercury. Submerge one of them in the water contained in the finger bowl and place the other in the empty finger bowl of the model for the students.

Quickly lift the pine branch and stopper out of the bucket and fit the stopper into the opening of the wide-mouthed bottle of the potometer. Don't squeeze the stopper tightly yet. Now work the end of the capillary tube filled with the boiled water into the second hole of the rubber stopper. It must fit snugly! Be sure the lower end of the capillary tube is in the water of the finger bowl. Now squeeze the rubber stopper tightly into the wide-mouthed bottle. As you squeeze you should force all of the air out of the capillary tube. *If there are any air bubbles left in any part of the system the potometer will not work properly. Remember we are depending on the cohesive force of water molecules to raise an uninterrupted column of water and mercury.*

If a bubble does appear or you wish to start the potometer over again, loosen the rubber stopper, fill the bottle once again with cool, previously boiled water, and squeeze the stopper back into place. If the mercury stays up in the capillary tube you can blow it out before you start the apparatus going. (CAUTION: *If students are working with mercury warn them not to get it on their hands or heat it and inhale the fumes.*)

Care must be taken not to let air into the end of the capillary tube when you move it into the submerged dish of mercury.

An electric fan blowing on the pine branch will hasten the rate of transpiration. Time the rise of the mercury column with and without the fan.

The student model of the potometer is the same as the potometer with the plant except that there is no water in the system. Any student who wants to should be given a chance to see how high he can raise the column of mercury by sucking on the mouthpiece at the end of the rubber tube. The inhaled air will come from the wide-mouthed bottle, which will trap any mercury that might by chance come all the way up the tube.

Have glass tubing mouthpieces with flamed ends standing in a dish of 70% alcohol. Each time a student tries to raise the column he should use a fresh mouthpiece from which the alcohol has been rinsed.

To flame the ends, heat them over a gas flame until they begin to melt. That will remove the rough edges.

After you have set up the potometer the first time, the procedure is easy to repeat. Leave the apparatus intact so that in the future all you will have to do is to collect new plant branches.

ANSWERS TO QUESTIONS

1. The pink color indicates that moisture is being given off by the leaf.
2. The presence of greater numbers of stomata on the lower surface promotes a greater water loss from that surface than from the upper one.

3. The pot with the plant.
4. The leaf surface for the evaporation of water in the pot with the plant allows for transpiration, which does not take place in the completely covered pot.
5. Water is absorbed by the roots, conducted to the leaves, and transpired to the outside through the stomata.
6. Loss of water molecules from cells by transpiration causes an increase in the concentration of dissolved materials within the cells. The water deficit is made up as water molecules are drawn in by diffusion from the veins. As the water supply in the vein is used, it pulls on the water absorbed by the roots, and raises water from the roots to the leaves.
7. Atmospheric humidity will reduce the rate of transpiration; the amount of light will affect the guard cells that regulate transpiration; an increase in temperature and air currents will speed the rate of evaporation; the amount of water available in the soil also affects the rate of transpiration.
8. The rate of transpiration would be greater on a dry, sunny, bright day, because of the factors listed in Question 7 above.
9. Conducting tissues (xylem) of roots and stem.
10. There is a continuous water column.
11. The water in the soil of the plant's natural environment.
12. An air bubble will break the cohesive force of water or mercury.
13. It ascends more rapidly than when the fan is off.
14. Because of the reduced rate of transpiration, the mercury column ascends more slowly.
15. Multiply the observed maximum height of the mercury column by 13.6 for the answer.
16. Pulled by transpirational pull.
17. Transpiration from the pine branch causes the water and mercury to move upward.
18. That force created by an attraction of like molecules of a substance for one another, as water molecules for water molecules, so that when a part of the column is moved the rest coheres and likewise moves.
19. Without cohesive force there would be no column of water to begin with, as the molecules would not attract one another and hold together.
20. Attraction between unlike molecules, that is, molecules of different nature.
21. Adhesion provides the force with which the water adheres to the walls of the tubes.
22. It depends on how high the student can pull the mercury times 13.6.
23. Cohesive force and transpirational pull, as outlined for Question 6.
24. Yes.

SUGGESTED READINGS

Books

Bonner, J., and A. W. Galston, 1952. *Principles of Plant Physiology.* W. H. Freeman, San Francisco

Curtis, O. F., and D. G. Clark. 1950. *An Introduction to Plant Physiology.* McGraw-Hill, New York

Kramer, P. J. 1959. "Transpiration and the Water Economy of Plants." *Plant Physiology.* Vol. 2, Academic Press, New York

Morholt, E., A. Joseph, and P. Brandwein. 1958. *A Sourcebook for the Biological Sciences.* Harcourt, Brace & World, New York

FILMS

Multicellular Plants, Part III: *Stems*, No. 7, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

In part, deals with cohesiveness of water molecules and transpiration in the leaf.

THE FLOWER

EXERCISE

17-1

TIME: One laboratory period.

MATERIALS: See page 131 in the *Student Laboratory Guide.*

This exercise is to accompany the section dealing with the flower in Chapter 17 of the textbook.

■ **The purpose of this exercise is to study the structure of a flower in relation to its reproductive functions.**

SPECIAL INSTRUCTIONS

This exercise provides a fine opportunity to strengthen the idea of complementarity of structure and function. The student will see in the flowering plants an ideally adapted reproductive structure (the flower) which has evolved as the most effective terrestrial reproductive device in plants.

At the conclusion of this exercise the student should have some understanding of how the seeds that he has studied and will study later are produced.

TECHNIQUES

If sweet pea flowers are not available *Gladiolus* flowers make a good substitute. These can be ordered from the local florist, but he should be called in ample time to make sure that he has an adequate stock. The other flowers used will depend very largely upon the florist's stocks. Each student should have a flower.

Flowers of *Vinca* produce pollen that will germinate in a matter of minutes. Flowers of this plant may be obtained from the local florist.

Demonstration slides of germinated pollen can be obtained from biological supply houses, if you wish to demonstrate germination.

Because this exercise may be done in the winter months when supplies of sweet pea flowers are difficult to obtain, other kinds of flowers, such as lilies and snapdragons, which are available in greenhouses, can be substituted. They have the same parts, arranged in a slightly different fashion. From previous exercises, you may have some bean plants in flower.

Each two students should be supplied with a fresh bean or pea pod.

ANSWERS TO QUESTIONS

1. Apparently they identify the flower to particular species of insects and serve to advertise the presence of nectar and pollen.
2. Pollen, nectar, or both.
3. In lily and *Gladiolus* the number is 6; it will vary with the flower the student is using.
4. Pollen may simply fall from the anther to the stigma. It can also be carried by insects, birds, or wind. In aquatic plants, water is the agent for pollination.
5. The student will have to count the little white structures inside the ovary of the flower he is using.
6. There must be some mechanism of getting to the egg through the ovary. When the student sees pollen tubes formed by pollen grains the answer to this question will be more obvious.
7. The pollen grain itself gets no closer to the egg than the surface of the stigma.
8. There must be a pathway that the sperm cells follow to the egg. The student will later note that the pollen grains grow pollen tubes in which the sperms are transported to the egg.
9. The ripened ovary. It is a fruit.
10. The ovule.
11. A pea or bean seedling. A new plant, indicating that sexual reproduction has been accomplished.
12. An embryo is formed in the seed. This little plant grows when the seed germinates.

SUGGESTED READINGS

Books

Frisch, K., von. 1953. *The Dancing Bees*. Harcourt, Brace & World, New York
Contains sections on bees and flowers.

Articles

Grant, V. 1951. "The Fertilization of Flowers." *Sci. Am.* 184 [6] 52
Naylor, A. W. 1952. "Control of Flowering." *Sci. Am.* 186 [5] 49

FILMS

Multicellular Plants, Part III: *Flower Structure*, No. 10, 1961, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Flower Functions*, No. 11, 1961, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

Flowers at Work, 1956, (sd, b&w or c, 11 min), p or r, EBF

The Dances of the Bees, 1950, (si, b&w, 22 min), Wilner

The Honeymakers, 1952, (sd, c, 20 min), Minnesota

A SIMPLE KEY TO FLOWERING PLANTS

EXERCISE
17-2

MATERIALS: See page 133 in the *Student Laboratory Guide*.

This exercise is to accompany the section on the flowering plant in Chapter 17 of the textbook.

■ **The purpose of this exercise is to acquaint the student with the use of a key as a mechanism for identifying and classifying unknown organisms and for emphasizing the important differences in general structural pattern among flowering plants.**

SPECIAL INSTRUCTIONS

The student should observe several of the important distinguishing differences in flowering plants through the use of a simple, dichotomous key of 17 common families (8 monocotyledonous and 9 dicotyledonous families).

Psychologically, the process of using a key provides a challenge or contest in which the student gains the satisfaction of accomplishment. By becoming familiar with a few key terms and by following directions carefully, he can demonstrate to himself his ability to identify correctly. Observation of structural differences and the mastery of a limited amount of terminology becomes for him a purposeful means of identifying rather than merely memorizing terminology. We have tried to reduce the necessary terminology to an absolute minimum.

Most of the families used in the key are represented by several common cultivated and wild species. Species used in this exercise should be selected with some care. A species chosen at random might belong to a family not included in the key, and then the key either will not fit at all or will lead the student to the wrong family.

TECHNIQUES

While not absolutely essential, the stereoscopic dissecting microscope or hand lens will be of great aid in identifying some of the smaller structures. The inclusion of scissors, scalpel, forceps, and dissecting needle is to aid in the necessary dissection of the flower parts which might not otherwise be seen.

It is suggested that a large-flowered species, such as *Gladiolus*, should be used at first to help students become acquainted with the key. The identification of six to ten species by family should provide enough experience for the purpose of this exercise.

Following is a list of the families used in the key, with certain wild and cultivated representative species.

MONOCOTYLEDONOUS FAMILIES

(* Indicates species which are likely to be common in the wild state, or which can be grown in the laboratory.)

Family	*Wild species*	*Cultivated species*
Amaryllis	Yellow star grass Atamasco lily Swamp lily (*Crinum*) **Agave*, century plant	**Amaryllis* **Narcissus* Daffodil (*Lycoris*) *Paper-white narcissus
Iris	Wild iris species Blue-eyed grass Blackberry lily	**Gladiolus* Crocus Cultivated iris
Grass	Any of numerous wild species	Wheat Rye *Oats Bluegrass Johnson grass
Lily	*Wild onion Wild lilies Cat brier Yucca Solomon's-seal	Onion Day lily Hyacinth Cultivated lilies Lily-of-the-valley
Sedge	*Nut grass Bullrush Spike rush Cotton grass Sedge	Egyptian paper plant (*Cyperus*)
Water Plantain	Water plantain (*Alisma*) Arrowhead (*Sagittaria*)	

Family	*Wild species*	*Cultivated species*
Spiderwort	*Spiderwort Roseling Dayflower	*Zebrina* *Rhoeo*
Orchid	*Spiranthes*, or any small-flowered wild orchid if abundant. (Most wild orchids should not be picked.)	*Any multi-flowered greenhouse orchid

DICOTYLEDONOUS FAMILIES

(* Indicates species which are likely to be common in the wild state, or which can be grown in the laboratory.)

Family	*Wild species*	*Cultivated species*
Buttercup	Buttercup Pasqueflower Marsh marigold Wild anemones	Peony (5-petaled) Christmas rose (*Helleborus*) Anemone
Composite	Black-eyed Susan Oxeye daisy Fleabane Wild aster *Gaillardia*	Dahlia Zinnia Chrysanthemum Shasta daisy Sunflower
Honeysuckle	Elderberry *Viburnum* Nannyberry Twinflower Japanese honeysuckle	Trumpet honeysuckle *Abelia* *Bush honeysuckle
Legume	Partridge pea Locust plant Wild indigo Hoary pea Locoweed Rattlebox	Sweet pea Wisteria Lupine Vetch Bean Clover
Mint	*Heal-all Skullcap Horsemint Henbit	Scarlet sage Blue sage Oswego tea Dragonhead
Mustard	*Shepherd's purse Pennycress Peppergrass Crinkleroot	Radish Water cress Mustard Turnip Stock

Family	*Wild species*	*Cultivated species*
Parsley	°Wild carrot	Dill
	Water parsnip	Parsley
	Angelica	Parsnip
	Water hemlock	Caraway
Phlox	Wild sweet William	Perennial phlox
	Blue phlox	°Drummond's phlox
	Gilia	Trailing phlox
	Jacob's-ladder	
Rose	Five-finger	Rose (5-petaled)
	Avens	*Spiraea*
	Wild rose	°Flowering quince
	Blackberry	Peach
	Hawthorn	Apple
		Plum
		Strawberry

SUGGESTED READINGS

Books

Cuthbert, M. J. 1943. *How to Know the Spring Flowers.* W. C. Brown, Dubuque, Iowa

______ 1948. *How to Know the Fall Flowers.* W. C. Brown, Dubuque, Iowa

Jaques, H. E. 1948. *Plant Families, How to Know Them.* W. C. Brown, Dubuque, Iowa

Wherry, E. T. 1948. *Wild Flower Guide.* (Northeast and Midland, U.S.) Garden City, New York

FILMS

Spring Blossoms, (sd, c, 19 min), Ott
Flowers at Work, 1956, (sd, b&w or c, 11 min), p or r, EBF
Mysteries of Plant Life, (sd, c, 20 min), Ball
Native Flowering Plants of California, (sd, c, 10 min), Bishop

SEEDS AND HOW THEY GROW

EXERCISE 17-3

TIME: One laboratory period.

MATERIALS: See page 139 in the *Student Laboratory Guide.*

This exercise is to accompany the section on the growth of seeds in Chapter 17 of the textbook.

■ **The purpose of this exercise is to study the seed and the rapid changes occurring during germination and seedling development.**

SPECIAL INSTRUCTIONS

The most dynamic aspects of plant growth are illustrated by the rapid changes occurring in seed germination and seedling development. The object of this exercise is to have the students see how well the development of parts is coordinated with the necessary functions of the plant. For example, the root is formed first, the leaves later, because the supply of stored food in the cotyledons allows the plant to become established before it is necessary to produce its own food by photosynthesis.

In order to accomplish this exercise it will be necessary to plant enough bean seeds 11 days in advance of the class period for this exercise, so that each student will have 10-day-old seedlings.

You must plant enough bean seeds 4 days in advance of class so there will be enough 3-day-old seedlings for one per student.

Start soaking the beans to be dissected one day before they are to be used in class, otherwise the exercise will be difficult, if not impossible.

"Invitations to Enquiry," No. 3, in the *Biology Teachers' Handbook* is on seed germination and deals with misinterpretation of data; it can be used at this time.

TECHNIQUES

You may want to have the students plant the bean seeds prior to class time and to bring them to the class in containers of vermiculite. It is possible to have each student plant bean seeds and take them home to observe them more closely for longer periods of time.

ANSWERS TO QUESTIONS

1. It protects the embryo from injury and from drying out completely.
2. The navel on our bodies is analogous to the scar of the seed coat.
3. As an embryo and fetus.
4. To supply food to the growing plant during the early stages of development.
5. The photosynthetic activity of the parent plant.
6. There is a crozier (crook) formed by the developing stem just below the cotyledons. This acts as a plow for clearing a path through the soil for the more delicate parts of the embryo.
7. The primary root.
8. This anchors the plant so it can support the stem and leaves and provides an immediate mechanism for absorbing large quantities of water from the soil.
9. The hypocotyl.
10. Hidden between the two halves of the cotyledon.

11. The epicotyl.
12. By this time the cotyledons on most of the seedlings have dropped off. If present, they are shriveled where they are attached to the stem.
13. It has been lost very early in development.
14. The epicotyl and the upper part of the hypocotyl.
15. Opposite one another.

SUGGESTED READINGS

Books

Ferry, J. F., and H. S. Ward. 1959. *Fundamentals of Plant Physiology*. Macmillan, New York

Machlis, L., and J. Torrey. 1956. *Plants in Action*. W. H. Freeman, San Francisco

FILMS

Multicellular Plants, Part III: *Development*, No. 8, 1961, (sd, c, 24 min), p or r, AIBS(McGraw-Hill)

Plant Growth, 1939, (sd, b&w, 11 min), p or r, EBF

Root Development, 1951, (sd, b&w, 10 min), p or r, UW

PLANT REACTIONS TO ENVIRONMENT

EXERCISE 17-4

TIME: One laboratory period. For Part A, an observation period 3 days after the start of the exercise. For Part B, an observation period 5 days after the start of the exercise.

MATERIALS: See page 141 in the *Student Laboratory Guide.*

This exercise is to accompany the section on environmental effects on plant growth and development in Chapter 17 of the textbook.

■ **The purpose of this exercise is to determine how plants react to certain factors in their environment. Part A is concerned with the effect of geotropism on the orientation of seedlings, Part B with the effect of phototropism on the growth of seedlings.**

SPECIAL INSTRUCTIONS

Both Parts A and B of this exercise can be done as a demonstration or assigned as separate projects to groups of students who later report the results to the class.

If Part A is to be used as a demonstration it should be started 5 days in advance of class. It serves as an excellent demonstration of the effect of gravity on the roots and shoots of plants.

"Invitations to Enquiry," No. 11, "Light and Plant Growth," in the *Biology Teachers' Handbook*, discusses the construction of hypotheses and may be introduced at this time.

TECHNIQUES

Large Petri dishes should be ordered ahead of time for this experiment. Their large size provides more room for growth of seedlings.

The cotton used for stuffing the Petri dishes should be of the nonabsorbent type.

Start corn grains soaking 1 day before the dishes are to be prepared. Be sure the dishes are packed tightly with cotton so the grains are held firmly against the glass when the dish is closed. Sealing the halves of the dish together is necessary to keep the top on and the seeds in place.

When the dish is placed in the vertical position, be sure to keep it from changing its orientation. Mark the dish with the word TOP by means of a wax pencil. The lower edge of the dish can be mounted in modeling clay to keep it from tipping over. In Part B, one should procure four wooden boxes that are light-tight. These boxes should be large enough to accomodate a small flower pot with germinating radish seedlings in it. Paint all four boxes black on the inside. Cut a rectangular hole in three of the boxes to admit light at a level just above the surface of the flower pot in which the seeds will be grown.

Cover the opening of one box with red cellophane, a second with blue.

Fill four 3-inch flower pots with soil and plant 20 radish seeds in each. Water, and put a pot under each of the four boxes.

The radish seedlings growing under the boxes should be ready for observation at the end of the fifth day. The seedlings will need to be watered once a day. Be sure that the positions of the pots in the boxes are not changed during watering until the observations are completed.

ANSWERS TO QUESTIONS

1. The uppermost grain has the shoot growing directly upward and the root growing directly down. Horizontally placed grains have shoots bending from the horizontal to an upright position, the roots emerging laterally and then bending downward. The grain at the bottom of the dish, which is upside-down, has the root emerging from the upper end and making a 180° bend so that the tip of the root is directed down. The shoot, on the other hand, emerges downward and then bends 180° so that the tip of the shoot is growing up.
2. Gravity.
3. The plants exposed to one-sided illumination by white light show the most obvious change in their direction of growth.

4. Plants exposed to illumination by blue light show bending toward the source of light; those exposed to red light show little or no bending toward the light stimulus.
5. The blue is far more effective than the red in causing bending.
6. There is much greater elongation when there is no light.
7. Presence of light may inhibit the formation of some substance that causes great elongation of cells.
8. Blue light has a greater effect than red in increasing stem elongation.

SUGGESTED READINGS

Articles

Ashby, E. 1949. "Leaf Shape." *Sci. Am.* **181** [4] 22
Bonner, J. T. 1956. "The Growth of Mushrooms." *Sci. Am.* **194** [5] 97
Schocken, V. 1949. "Plant Hormones." *Sci. Am.* **180** [5] 40
Weiss, F. J. 1952. "Chemical Agriculture." *Sci. Am.* **187** [2] 15
White, P. R. 1950. "Plant Tissue Cultures." *Sci. Am.* **182** [3] 48

FILMS

Multicellular Plants, Part III: *Regulation of Growth*, No. 9, 1961, (sd, c, 26 min), p or r, AIBS(McGraw-Hill)
Roots of Plants, 1931, (sd, b&w or c, 11 min), p or r, EBF
Sensitivity of Plants, 1957, (sd, b&w, 10 min), p or r, Almanac
Plant Oddities, 1950, (sd, c, 10 min), IFB
Plant Survival, 1953, (sd, b&w, 11 min), p or r, UW

REGULATION OF GROWTH IN PLANTS

EXERCISE 17-5

TIME: One period; or as a homework assignment.

MATERIALS: None

This exercise is to accompany the section on plant growth in Chapter 17 of the textbook.

■ **The purpose of this exercise is to have the students analyze experimental data given them and to draw conclusions regarding the role of auxins in the regulation of growth in plants.**

SPECIAL INSTRUCTIONS

Plants in your classroom that are bending toward the light will serve to introduce the experiment. After introducing the reaction of plants to light, turn to the exercise. The students may either work through the exercise in class or at home. *When they have completed the exercise, discuss the answers with the class.* Several purposes may be served by the use of this exercise for average and better-than-average students. First, it will emphasize that the often-heard expression "so and so happens because the plant (or animal) needs it" does not explain the mechanism involved. Second, the exercise will illustrate how a series of simple but well-designed experiments can be used to discover some of the steps which operate in a particular process or reaction. With some guidance, it will challenge the student to work out for himself a reasonable interpretation of the mechanism involved in this study. Finally, it will help the student to develop an understanding of the hormone concept in plants and to see how auxins may be involved in regulating plant growth.

Specifically, this exercise should lead the student to his own conclusions that something from the tip influences growth (elongation) below the cut (Experiment 1), and that this something is soluble in water since it will diffuse through an agar block (Experiment 2). The student will discover that it is not the tip itself but something that can be extracted from the tip (Experiment 3); and that if this something (perhaps the student can now be introduced to the term *auxin*) is provided to only one side of the plant, that side will grow more than the side without it, causing bending (Experiment 4). In Experiment 4, probably only the more competent students will be able to devise the hypothesis—that bending is dependent upon a chemical substance and that light may influence the formation or activity of this substance. Now the student has enough information to analyze the results of Experiment 5, and thus hypothesize that Plant N in Experiment 5 bends, because the light apparently destroys the growth substance on the side toward the light. It may be assumed that Plant O does not grow and bend because the auxin is destroyed on the right side and prevented from reaching the cells below on the left side. Question 21, Experiment 5 is purposefully ambiguous. In our experience, the vast majority of students answer "No." "Yes," followed by an explanation that it is to the plant's advantage to bend toward the light must certainly be considered correct if the student understands the evolutionary basis of the mechanism and does not assign conscious purpose to the plant.

"Invitations to Enquiry," No. 14, "Auxins and Plant Movement," and No. 30, "Light and Auxin Formation," in the *Biology Teachers' Handbook*, are appropriate at this point.

ANSWERS TO QUESTIONS

1. Below.
2. Coleoptile C with the tip removed showed no elongation. This means that the tip produces something necessary for elongation.
3. B had the tip replaced; C had it removed.
4. As a control.
5. There is something produced in the tip of the stem that stimulates elongation below the cut.
6. They differed in the nature of the sheets of material used to separate the tip from the lower part of the coleoptile. Water and other substances in solution can diffuse through the agar block of Plant E, but the mica forms an impervious layer between the tip and the rest of the coleoptile in Plant F.
7. As a control.
8. Judging from the growth accomplished by Plant E and the lack of growth of Plant F, we can conclude that the substance produced by the tip is water-soluble and will diffuse through the agar.
9. In the composition of the agar block. That of Plant H has the juice extracted from the tips of the coleoptiles, while that of Plant J lacks this substance.
10. To make sure that it was not some substance in the agar that caused the elongation of the coleoptile. It is another control.
11. The tip is not required for elongation. The experiment shows that it is a substance produced by the tip that is required.
12. On the same side as the block containing the juice from the tip. This was the left side, so it curved toward the right.
13. It was the right side of Plant M where the greatest elongation occurred, so it curved toward the left.
14. The substance in the juice from the tips applied to only one side of the cut coleoptile will cause elongation on the side where it is applied.
15. The light might have some inhibiting influence on the formation or activity of the growth substance, so that the greater elongation of the coleoptile occurs on the side away from the light.
16. Some substance produced by the growing tip.
17. All were grown in the dark.
18. The effect of light is to cause the stem tip to bend toward the light.
19. Because of an unequal distribution of auxin.
20. Plant O failed to grow because the activity of the growth substance was inhibited by the light on one side, and its distribution from the tip to the elongating region was prevented on the other side by the piece of mica.
21. No.
22. The plant bends toward light because of an unequal distribution of the growth substance in the growing tips of the stem. The unequal distribution is affected by light which inhibits the formation of growth substance on the illuminated side of the stem.

SUGGESTED READINGS

Articles

Greulach, V. A. 1955. "Plant Movements." *Sci. Am.* **192** [2] 100

Schocken, V. 1949. "Plant Hormones." *Sci. Am.* **180** [5] 40

FILMS

Multicellular Plants, Part III: *Development*, No. 8, 1961, (sd, c, 24 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Seeds and Germination*, No. 2, 1961, (sd, c, 24 min), p or r, AIBS(McGraw-Hill)

Multicellular Plants, Part III: *Leaves*, No. 3, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Plant Growth, 1939, (sd, b&w, 11 min), p or r, EBF

Animals

The first six basic exercises utilize the single-celled *Paramecium* as an experimental animal. It is possible, with this simple organism, to demonstrate all the major features of animals. Because it is always available, cheap, small, easily seen under the microscope, and capable of being experimented with, it has been selected for our introduction to animals.

Just as the introduction to cells used the experimental method of deriving a generalized cell from a series of observations, so do the exercises on *Paramecium* ask the student to observe, formulate hypotheses concerning structure and function, and then test these hypotheses in a series of simple experiments leading to conclusions about the animal way of life.

We recommend that these exercises start early so that the student will have completed them by the time he has discovered the sections on *Paramecium* in the textbook.

Following the generalizations about animals, there are four exercises in which the students can compare and contrast a variety of multicellular forms with the *Paramecium* just studied. The first exercise (20-1), contrasts a free-living and a parasitic worm. This exercise should enable the student to understand adaptation to free-living and parasitic ways of life.

Exercise 20-2 is concerned with the highly variable and adaptive arthropods and Exercise 20-3 briefly covers that classic vertebrate, the frog. Exercise 20-4, dealing with the principles of animal classification, gives the teacher the opportunity to introduce a variety of animals. In this exercise, the keys have been made simple deliberately for ease of use. However, it should constantly be brought to the attention of students that the simpler the key, the less accurate it will be for any given group of animals. To emphasize this, the teachers may wish to introduce more elaborate and detailed keys for species of common animals of the local area.

Exercises 21-1 through 26-1 elaborate physiological processes in multicellular animals. They cover the processes and structures of digestion, circulation, respiration, excretion, nervous and hormonal integration, and muscle contraction.

Exercises 27-1 through 28-2 are specifically concerned with reproduction and development, and provide simple, classical, dramatic examples through which the student can understand the processes of reproduction and development.

The exercises on development require rather large numbers of living animals, especially frogs and fertilized chick eggs.

Certain equipment not ordinarily found in the high school laboratory may also be necessary. For example, previous experience has shown that teachers may obtain old refrigerators and convert them into incubators for chick eggs. An ordinary household refrigerator is also handy for holding frogs and for keeping frog eggs at low temperatures (20° C is a good average). The incubator will also be useful in the section on genetics, for which purpose it is set at approximately 25° C. For use with chick eggs it should be held at approximately 38° C and be adequately humidified.

Instruments used for work with living embryos should preferably never have been used with solutions containing formalin; the same is true of glassware and other materials which will come in contact with solutions containing the eggs. Formalin is extremely toxic to living organisms, especially in embryonic stages, and is very difficult to remove from instruments.

Much help can be obtained from *A Manual of Experimental Embryology*, revised edition, by Viktor Hamburger, University of Chicago Press.

The BSCS laboratory block "Animal Growth and Development" will also prove helpful, and several of the exercises are quite similar to portions of that block, which was written by Dr. Florence Moog.

Exercise 29-1 deals with problems of regeneration and growth, and here, again, the student will repeat classical experiments which demonstrate beautifully the phenomenon of regeneration.

You may miss many old and familiar experiments in this animal section. However, the inclusion of some exercises and the exclusion of others is in keeping with the BSCS philosophy. No attempt has been made to emphasize morphology and systematics, but rather to use them to achieve for the student the concept of the functioning animal as an integrated organism. Exercise 20-3 on the frog may lead you to desire more latitude in time and terminology. However, we urge you not to expand the terminology and morphology of this exercise, which is far less dramatic and interesting to students, and which will take much needed time from the exciting material of genetics, evolution, and ecology, yet to come.

STRUCTURE AND FUNCTION IN *PARAMECIUM*

EXERCISE 19-1

TIME: One laboratory period.

MATERIALS: See page 149 in the *Student Laboratory Guide*.

This exercise is designed to introduce the student to the animal way of life and should accompany Chapter 18 in the textbook.

■ **The purpose of this exercise is to emphasize the theme of complementarity of structure and function as seen in animals. The student is asked to correlate structures with their presumptive functions, both of which are as yet unknown to him.**

SPECIAL INSTRUCTIONS

Exercises 19-1 through 19-6 have an investigatory aspect which is realized only by completion of all six as a unit. In order to provide time in which to accomplish this, no exercises are presented for Chapter 18. It would be desirable, therefore, to do Exercise 19-1 just as the student begins Chapter 18. The purpose of these six exercises is to allow the student, from his own laboratory observations, to create a typical animal in his own mind. In this exercise he is making generalized hypotheses. In subsequent exercises he will confirm his observations and test his hypotheses, until he ends up with a concept of a complete simple animal.

It is advisable that the student complete this series of exercises before reading Chapter 19, in order to preserve the investigatory flavor.

This plan for studying the *Paramecium* in the laboratory is similar to that for the development of the generalized cell in the exercises relating to Chapter 3.

It is possible to utilize this sequence of exercises while doing Chapter 19, but the chance for the student to find things out for himself, without reliance on the textbook, would thereby be lost.

Do not at this time be concerned with the labeling of the drawings. It will be sufficient for the student to indicate the functions of the structures he sees. In subsequent exercises he will learn the names of the structures he has labeled, along with their functions.

TECHNIQUES

It is important that the student make the ring of methyl cellulose approximately the diameter of a lead pencil and place the drop with the paramecium in the center of the ring before adding the cover glass. It may take as long as 15 minutes for the methyl cellulose to diffuse through the water and to slow down the paramecium. However, many of the desired observations can be made before the methyl cellulose takes effect.

In lieu of methyl cellulose, teachers have had success in using fine wisps of cotton, the fibers of which entrap the paramecia. A piece of lens paper also provides a source of fibers when pulled apart and placed on the slide in a drop of water.

ANSWERS TO QUESTIONS

1. It is elongated and somewhat slipper-shaped. For this reason it is sometimes referred to as the "slipper animalcule."
2. Relatively rapidly, in relation to their own size.

3. This depends largely upon the amount of light being used. The diaphragm should be closed and opened to vary the amount of light until the student has seen most of the structures. He will not see the macronucleus and micronucleus, however.
4. The staining has made the macronucleus visible. (The student will perhaps also see the micronucleus.)
5. Depending on the depth of stain and the condition of the animal at the time it was killed, all things could be seen that were seen in the living animal plus the macronucleus and micronucleus. However, if both contractile vacuoles were completely contracted, they would not be seen after staining.
6. The living organism. Function is not observable in a dead one.
7. Coordination (nervous or endocrine), reproduction, reception of stimuli, secretion, chemical synthesis, and growth.
8. No. There would not be a hydrostatic organ, nor movement for the most part, nor special sites of food intake, nor a discharge of undigested food remains, nor special attention to excretion of liquid wastes.
9. It is capable of taking in large food molecules, digesting them, and discharging undigested food; it is capable of respiration and movement and excreting liquid wastes; it is capable of maintaining a hydrostatic balance between itself and the environment.

SUGGESTED READINGS

Books

Wichterman, R. 1952. *The Biology of* Paramecium. McGraw-Hill, New York

Hyman, L. 1940. *The Invertebrates: Protozoa through Ctenophora.* Vol. 1. McGraw-Hill, New York

——— 1959. *The Invertebrates: Smaller Coelmate Group.* Vol. 5. McGraw-Hill, New York

Chapter 23 has a review of more recent work on the protozoa than is represented in Vol. 1.

Makinnon, D. L., and R. S. J. Hawes. 1961. *An Introduction to the Study of Protozoa.* Oxford Univ. Press, London.

Chapter 5 deals with methods in protozoology, including collection and culturing.

FILMS

Life in a Cell, 1960, (sd, c, 14 min), p or r, Colburn

Uses the amoeba as an example of the seven functions of life.

Microbiology, Part II: *The Very Small,* No. 1, 1961, (sd, c, 27 min), p or r, AIBS (McGraw-Hill)

Microbiology, Part II: *Complex Microorganisms,* No. 3, 1961, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

MOVEMENT AND *PARAMECIUM*

EXERCISE 19-2

TIME: One laboratory period.

MATERIALS: See page 151 in the *Student Laboratory Guide.*

This exercise is designed to accompany the sections on animals and locomotion in Chapter 19 of the textbook.

■ **The purpose of this exercise is to examine locomotion by cilia in a paramecium and to compare and to contrast it with animal locomotion in general.**

SPECIAL INSTRUCTIONS

Remember that the purpose of this exercise is the observation of movement. Even though the carmine particles used may be ingested by the paramecium, this is not to be the focal point of this exercise.

The student will, from this exercise, be able to go back and reconsider the hypothesis about movement that he formed in Exercise 19-1. This will allow him to review general animal characteristics outlined in Exercise 19-1 and will allow him to make cogent observations relating to locomotion in animals.

TECHNIQUES

Students get so absorbed in watching the paramecium that the slides sometimes dry out. Caution them to add water from time to time to their microscope preparation. This will prevent the animals from being destroyed by dry conditions. A very small amount of carmine powder in solution will be sufficient for this exercise.

In order to observe cilia properly the student will have to regulate the amount of light by adjusting the diaphragm on his microscope. Too much light will make it impossible to observe the movement of cilia. A yeast suspension or India ink may be used in lieu of carmine.

Exercises 19-1 and 19-2 can be combined and accomplished in one laboratory period.

ANSWERS TO QUESTIONS

1. The student will most likely think of sponges. Any sessile animal such as bryozoa, sporozoa, hydra, oysters, etc. will be suitable examples.
2. Yes.
3. Yes. One end, the anterior, is more rounded; the other, the posterior, is more pointed.
4. Because it has the general shape of a slipper.
5. Yes, slightly.
6. No.

7. It rotates as it moves forward or backward.
8. It backs up, changes direction slightly, and moves forward again. It continues this process until it by-passes the obstacle.
9. A trial-and-error method of behavior suggests a relatively simple behavioral mechanism.
10. They act like tiny oars to move the animal.
11. Since the carmine particles move by in one direction, the cilia must also move in a common pattern. They beat strongly and swiftly in one direction, more slowly and weaker in the opposite direction.
12. Individual cilia can be seen with proper lighting.
13. From this exercise the student should determine that the cilia serve to move the paramecium.
14. There is a coordinating mechanism to move the paramecium through the medium in which it lives. This type of coordinated locomotion is necessary whenever animals are to move.

SUGGESTED READINGS

Articles

Allen, R. D. 1962. "Amoeboid Movement." *Sci. Am.* **206** [2] 112
Astbury, W. T. 1951. "Flagella." *Sci. Am.* **184** [1] 20
Hayashi, T. 1961. "How Cells Move." *Sci. Am.* **205** [3] 184
This entire issue is devoted to cells.
Satir, P. 1961. "Cilia." *Sci. Am.* **204** [2] 108

FILMS

Microbiology, Part II: *Responses in Motile Forms*, No. 5, 1961, (sd, c, 25 min), p or r, AIBS(McGraw-Hill)
Amoeba, 1935, (sd, b&w, 11 min), p or r, UW

INGESTION AND DIGESTION IN *PARAMECIUM*

EXERCISE

19-3

TIME: One laboratory period.

MATERIALS: See page 153 in the *Student Laboratory Guide.*

This exercise is to accompany the material on ingestion and digestion in Chapter 19 of the textbook.

■ **The purpose of this exercise is to determine how *Paramecium* ingests food, digests it, and egests the undigested residue.**

SPECIAL INSTRUCTIONS

The yeast suspension should be prepared the day before class. Just before class the mixture can be boiled and the Congo red powder added before staining.

Call attention to the resemblance between the streaming of cytoplasm within the *Paramecium* and cyclosis, which the student has seen in plant cells.

Focus the attention of the student on the ingestion–digestion–egestion of food particles. By this time the students should be very familiar with *Paramecium* as an experimental animal and should be able easily to observe the reactions.

TECHNIQUES

The ring of methyl cellulose is essential in order to slow down the animal sufficiently for observation.

Congo red is red at a pH of 5 and blue at a pH of 3. It will change from red to blue in the vacuoles in approximately 2 minutes, so the observations will have to be made rapidly.

P. multimicronucleatum is recommended as a species of *Paramecium* large enough to be observed easily.

ANSWERS TO QUESTIONS

1. The yeast cells are red or pink.
2. They have been dyed by the Congo red.
3. They enter the oral groove and move down the gullet.
4. The food vacuoles are formed at the base of the gullet.
5. The color turns from red to blue.
6. There may be a return to the red color as the contents of the food vacuole change pH again.
7. The change to a pH of approximately 3 kills the yeast cells or other food and begins the process of digestion. In the acidic medium, certain enzymes will operate. Later, the pH will become basic and a different set of enzymes will start working.
8. They move slightly backward from their point of origin, then pass anteriorly in the animal in the streaming cytoplasm, returning in about one hour to a point (the anal pore) near where they were formed and the undigested food contained in the vacuole is egested, i.e. ejected to the outside.
9. It is localized at the anal spot, not far from where the food vacuoles were originally formed.
10. Food is swept into the gullet by means of the cilia and a small food vacuole forms at the base of the gullet; the vacuole is then set free in the cytoplasm. In this vacuole, digestion proceeds first in an acidic, later in a basic, medium. Assimilation occurs during the movement of

the food vacuole, and the undigested food residues are discarded to the outside at a spot near the place where the vacuole was originally formed. The general process involved is not unlike that in a human being. The structures and the details differ, however.

SUGGESTED READINGS

Articles

Green, D. E. 1949. "Enzymes in Teams." *Sci. Am.* **181** [3] 48
Linderstrom-Lang, K. U. 1953. "How Is a Protein Made?" *Sci. Am.* **189** [3] 100
Meyerhof, O. 1950. "Biochemistry." *Sci. Am.* **183** [3] 62
Pfeiffer, J. E. 1948. "Enzymes." *Sci. Am.* **179** [6] 28

FILMS

The Multicellular Animal, Part IV: *Ingestion and Digestion*, No. 3, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)
Digestion (Chemical), 1949, (sd, b&w or c, 19 min), p or r, UW
Digestion of Foods, 1938, (sd, b&w, 11 min), p or r, EBF
Digestion of Foods, 1948, (fs, b&w, 70 fr), p or r, EBF

CONTRACTILE VACUOLES IN *PARAMECIUM*

EXERCISE 19-4

TIME: One laboratory period.

MATERIALS: See page 155 in the *Student Laboratory Guide.*

This exercise is designed to precede the section on homeostasis in Chapter 19 of the textbook.

■ **The purpose of this exercise is to locate the contractile vacuole and to observe its activity. The student will be asked to draw conclusions from his own observations and the observations of others regarding the function of the contractile vacuole.**

SPECIAL INSTRUCTIONS

The theme of homeostasis can be emphasized here as the student comes to realize that the contractile vacuoles serve a hydrostatic function. The regulation of water balance in paramecia is for it as important a function as respiration or digestion. Students frequently are led to the erroneous conclusion that the contractile vacuoles are solely excretory organs, without realizing the necessity for the maintenance of water balance.

This exercise is a continuation of the investigatory series on a simple unicellular animal and should be completed before the student reads that portion of the text on the contractile vacuole.

TECHNIQUES

Chinese ink, obtainable in stick form from artists' supply houses, can be dissolved in the water to show the contractile vacuoles, which tend to concentrate it.

The contractile vacuole can be better observed if some of the water is withdrawn from under the cover glass so that the animal is flattened. However, care must be used so that the animal does not dry out.

ANSWERS TO QUESTIONS

1. Two.
2. One at the anterior, one at the posterior part of the body.
3. They stay in one place.
4. Clear.
5. Empty.
6. The number varies from 5 to 12.
7. The number of contractions varies greatly with environmental conditions but averages about three per minute.
8. Depends upon the students' response.
9. Because so little urea has been found in the contractile vacuole, it would be dubious to consider it solely an excretory organ.
10. It functions as a structure to rid the organism of excess water.
11. Through the cell membrane or in the food vacuoles.
12. The cell membrane must be the main passage for water into the body.
13. The contractile vacuole is a hydrostatic organ.
14. Placing the paramecium in different concentrations of salts and observing the action of the contractile vacuole. The greater the salt concentration, the less active should be the contractile vacuole, and vice versa.

SUGGESTED READINGS

Books

Wichterman, R. 1952. *The Biology of* Paramecium. McGraw-Hill, New York

FILMS

Paramecium, 1950, (sd, b&w, 11 min), p or r, UW

BEHAVIOR OF *PARAMECIUM*

EXERCISE
19-5

TIME: One laboratory period.

MATERIALS: See page 157 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on animal behavior in Chapter 19 of the textbook.

■ **The purpose of this exercise is to observe the responses of paramecia to a number of different environmental stimuli and to formulate some hypotheses regarding behavior in *Paramecium*.**

SPECIAL INSTRUCTIONS

This exercise may be the students' first contact with the phenomenon of behavior and the terminology incidental to behavioral studies. Therefore, you may wish to take some time to acquaint the student with such terms as *instinctive behavior, learned behavior, taxis, insight, memory,* and *intelligent behavior.*

Despite claims in the literature regarding conditioned reflexes, associations, and true learning in *Paramecium*, it is best to consider this animal as reacting purely in a trial-and-error fashion. Some of the work attempting to demonstrate learning in *Paramecium* involves faulty techniques and failure to understand the general physiological activities of the organism under different conditions.

TECHNIQUES

This exercise works well either with teams or with individual students. The concentration of HC1 to be used here can be made by putting 1 drop of the acid in 20 drops of water.

The reversal switch is optional.

Many of the responses described in this exercise can be demonstrated with a microprojector to the class as a whole.

ANSWERS TO QUESTIONS

1. At the upper end of the tube.
2. Negatively geotactic.
3. Yes. Oxygen is a favorite answer, although light, food, pH, and other factors may be mentioned.
4. Depends on the answer to Question 3. All other variables except gravity must be eliminated. Oxygen could be ruled out if the tube were filled completely with water, tightly corked, and shaken before obser-

vation; light could be eliminated or controlled; pH could be tested; food could be evenly distributed throughout the tube.

5. The uncovered or lighted end.
6. Positively phototactic.
7. Because the reaction of *Paramecium* then would perhaps be confused with its reaction to gravity.
8. The food it consumes may need light. In addition, some paramecia contain chloroplasts (on account of symbiotic algae) and require light for photosynthesis.
9. Yes.
10. Positively galvanotactic.
11. They concentrate primarily at one pole.
12. The negative pole.
13. Yes. They leave the pole which is not positive and go toward the negative one.
14. Positive chemotaxis.
15. A negative chemotactic response.
16. No evidence of learned behavior can be seen in this experiment.
17. Yes, because it responds in a similar fashion each time.
18. Some coordination is necessary to make the cilia beat in unison and in a common direction, even though no such system can be seen without special microscopic methods.
19. The student's answer will probably include the statement that *Paramecium* seems incapable of learning or of any other cerebral activity, in contrast to the cat or dog. The major idea is to elicit an answer where the student sees some similarities between the behavior of paramecia and that of higher animals, as well as the differences in behavior.

SUGGESTED READINGS

Books

Wichterman, R. 1952. *The Biology of* Paramecium. McGraw-Hill, New York
Chapter 8 deals with the behavior of *Paramecium*.

Articles

EXPERIMENTS IN ANIMAL BEHAVIOR:
Evans, R. M. 1949. "Seeing Light and Color." *Sci. Am.* **181** [2] 52
Gray, G. W. 1955. "The Yerkes Laboratories." *Sci. Am.* **192** [2] 67
Milne, L. J., and M. J. Milne. 1950. "Animal Courtship." *Sci. Am.* **183** [1] 52
Olds, J. 1956. "Pleasure Centers in the Brain." *Sci. Am.* **195** [4] 105
Skinner, B. F. 1951. "How to Teach Animals." *Sci. Am.* **185** [6] 26
Sperry, R. W. 1956. "The Eye and the Brain." *Sci. Am.* **194** [5] 48
Strecker, R. L. 1955. "Populations of House Mice." *Sci. Am.* **193** [6] 92
Thompson, W. R., and R. Melzack. 1956. "Early Environment." *Sci. Am.* **194** [1] 38
Witt, P. N. 1954. "Spider Webs and Drugs." *Sci. Am.* **191** [6] 80

ANIMAL INTELLIGENCE:
Best, J. B. 1963. "Protopsychology." *Sci. Am.* **208** [2] 54

Guttman, N. and H. I. Kalish. 1958. "Experiments in Discrimination." *Sci. Am.* **198** [1] 77
Holst, E., von and U. von St. Paul. 1962. "Electrically Controlled Behavior." *Sci. Am.* **206** [3] 50
Munn, N. L. 1957. "The Evolution of Mind." *Sci. Am.* **196** [6] 140
Pastore, N. 1955. "Learning in the Canary." *Sci. Am.* **192** [6] 72
Rensch, B. 1957. "The Intelligence of Elephants." *Sci. Am.* **196** [2] 44

ANIMAL BEHAVIOR IN NATURE:
Gilmore, R. M. 1955. "The Return of the Gray Whale." *Sci. Am.* **192** [1] 62
Hess, E. H. 1958. "Imprinting in Animals." *Sci. Am.* **198** [3] 81
Leopold, A. S. 1955. "Too Many Deer." *Sci. Am.* **193** [5] 101
Lack, D., and E. Lack. 1954. "The Home Life of the Swift." *Sci. Am.* **191** [1] 60
Minton, S. A., Jr. 1957. "Snakebite." *Sci. Am.* **196** [1] 114
Nicholas, Bro. G., F. S. C. 1955. "Life in Caves." *Sci. Am.* **192** [5] 98
Thorpe, W. H. 1956. "The Language of Birds." *Sci. Am.* **195** [4] 128

FILMS

Analysis of the Forms of Animal Learning, Parts I and II, 1940, (si, b&w, 17 min), r, Pennsylvania
Cell Biology, Part I: *Responsiveness*, No. 8, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)
Paramecium, 1950, (sd, b&w, 11 min), p or r, UW

REPRODUCTION IN *PARAMECIUM*

EXERCISE
19-6

TIME: One laboratory period.

MATERIALS: See page 159 in the *Student Laboratory Guide.*

This exercise is to precede the section on reproduction in Chapter 19 of the textbook.

■ **The purpose of this exercise is to acquaint the student with asexual and sexual reproduction in a simple animal, *Paramecium.***

SPECIAL INSTRUCTIONS

This exercise will constitute the student's introduction to animal reproduction. No opportunity should be lost to compare the processes seen here with those previously seen in microorganisms and in plants. Constant emphasis should be placed on the unity of reproductive processes throughout the living world.

Both sexual and asexual reproductive processes are demonstrated in

Paramecium as well as in other animals. The essential features are similar throughout the animal kingdom, however.

Emphasis should be placed on the genetic consequences of sexual versus asexual reproduction. The student should understand that asexual reproduction produces only individuals similar to the parental type and that sexual reproduction alone allows for any recombination of nuclear materials and production of numerous hereditary types that will be of selective value in the process of evolution.

It is preferable to use living cultures for this exercise. However, if it is not possible to obtain living cultures for demonstrating conjugation and fission, prepared slides may be used.

It will be necessary to explain mating types to the student, since no sexual dimorphism occurs in *Paramecium*. The student should understand that conjugation takes place between individuals that differ from one another in physiological ways which are not noticeable structurally. Mating types will conjugate only with definite other mating types, and conjugation may not occur between any two individuals of the same mating type.

In the demonstration of mating types, the paramecia are first seen to clump, and then to conjugate in pairs. Temperature and time of day influences conjugation. Without conjugation the particular strain dies out after approximately 350 generations.

TECHNIQUES

If cultures of paramecia are available, conjugation can be induced by starvation following rich feeding (Giese, A. 1935. *Physiological Zoology*, 8). Similarly, crowding large numbers of paramecia in a small dish of fresh medium will induce conjugation. (Sonneborn, T. M. 1936. *Genetics*, **41.**) The addition of certain salts, such as ferric and aluminum chlorides, will also induce conjugation. (Zweibaum, J. 1912. *Arch. Protistk.*, **26**; *Idem*, 1921-22:**44.**)

ANSWERS TO QUESTIONS

1. No, it is somewhat elongated.
2. The macronucleus is much elongated from anterior to posterior, and may be constricted in the center. The micronucleus, if seen at all, has already divided and the daughter nuclei have moved to opposite ends of the animal.
3. The micronucleus is difficult to see. However, there may be one or more micronuclei present, depending upon what species of *Paramecium* is being used.
4. Two individuals will be produced.
5. As both are identical, neither can be called a parent, any more than either one can be called an offspring of the other.
6. The budding seen in yeasts; fission in bacteria.
7. As it involves an increase in the number of cells, it is a process of cell-

ular reproduction. However, it is not reproduction, in the sense of an origin of new individuals.

8. Asexual.
9. Because there is no exchange of nuclear material between the animals—no meiosis and fertilization.
10. In conjugation, the two animals are side by side; in fission, the constriction produces the appearance of two smaller animals end to end.
11. No. The macronucleus loses its typical shape, moves from its usual position, and breaks up into pieces, spherical or sausage-shaped, that are ultimately reabsorbed by the cytoplasm. The micronuclei increase in size, and take on the characteristic shape of a crescent or sickle. They then go through the two meiotic divisions.
12. There is apparently a nuclear reorganization and exchange.
13. No, they are similar in appearance.
14. Sexual.
15. Because there is meiosis followed by an exchange of nuclear materials between individuals, so that the new individuals have a biparental heredity.
16. Sexual reproduction.
17. Because it allows for variation in the genetic make-up of the *Paramecium* through recombination, and this variation may be advantageous in a changing environment.
18. Functions can sometimes be localized by removing the suspected structure and watching the behavior of the animal subsequently.
19. A complex animal.
20. Because of the fact that it carries on all the life processes in a highly compact cell.
21. Because it reproduces rapidly and is easily maintained in the laboratory, and large numbers are available for experimental work. In addition, its habit of reproducing asexually produces large numbers of individuals of identical genetic constitution with which to experiment, and thus reduces the variability occasioned by sexual reproduction.

SUGGESTED READINGS

Books

Wichterman, R. 1952. *The Biology of* Paramecium. McGraw-Hill, New York
Chapter 9 deals with reproduction.

Articles

Beadle, G. W. 1948. "The Genes of Men and Molds." *Sci. Am.* **179** [3] 30
Bonner, J. T. 1949. "The Social Amoebae." *Sci. Am.* **180** [6] 44
______ 1950. "Volvox, a Colony of Cells." *Sci. Am.* **182** [5] 52
Gray, G. W. 1957. "The Organizer." *Sci. Am.* **197** [5] 79

FILMS

Paramecium, 1950, (sd, b&w, 11 min), p or r, UW

Continuity of Life Series: *Asexual Reproduction*, 1954, (sd, b&w or c, 10 min), r, Indiana

Principles of Genetics, No. 35: *Nucleo-Cytoplasmic Relations in* Paramecium, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Cell Biology, Part I: *Cell Reproduction,* No. 11, 1960, (sd, c, 39 min), p or r, AIBS (McGraw-Hill)

Microbiology, Part II: *Reproduction,* No. 7, 1961, (sd, c, 26 min), p or r, AIBS (McGraw-Hill)

STATE INSTITUTE OF EDUCATION
Govt. of West Bengal.

EXERCISE 20-1

PARASITIC AND FREE-LIVING WAYS OF LIFE

TIME: Two laboratory periods.

MATERIALS: See page 161 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on the phyla Nematoda and Annelida in Chapter 20 of the textbook.

■ **The purpose of this exercise is to study the differences between free-living and parasitic worms and the differences between worms of the phylum Nematoda and those of the phylum Annelida.**

SPECIAL INSTRUCTIONS

The essential part of this exercise is the observation of living materials – the earthworms and vinegar eels. Emphasis, therefore, should not be on the dissections but on the observations of living materials. By his observations of free-living and parasitic worms, the student should be able to contrast the structure and function of bodily parts and relate them to the mode of existence of each worm.

This exercise can be done in one period if the internal anatomy is shown as a demonstration.

Students should be introduced to the general terms which will be used extensively in the animal exercises. These include *dorsal*, *ventral*, *anterior*, and *posterior*.

TECHNIQUES

Be sure to have both male and female ascarid worms on display. Caution the students in handling *Ascaris*. The eggs of *Ascaris* are viable even after being soaked in formaldehyde for a considerable length of time. They should be handled with forceps or needles and the hands thoroughly washed after handling them.

If time allows, the student may wish to do the dissections of *Ascaris* and earthworm as outlined in the student guide. However, if time is at a premium, then you should have prepared earthworm dissections pinned out and covered with water for student observation. Certain students may be assigned to do these dissections before class as a special project.

Be sure that the students take a small drop of culture from the bottom of the vinegar eel container. The tendency is to take too large a drop and from too near the surface so that it contains few, if any, vinegar eels.

While the cross section slides of earthworm and *Ascaris* are not absolutely essential, they are recommended to give the student an understanding of the musculature and the internal anatomy of both forms.

The formulation of the hypotheses regarding the structures of various systems in *Ascaris* might well be done in classroom discussion, leading the students to a comprehension of the fact that these systems do reflect the way of life of the animal.

Ascaris can be used as a starting point for a discussion of parasitism among animals in general.

ANSWERS TO QUESTIONS

1. Vinegar eels are rapid-moving.
2. They whip from side to side apparently in random fashion.
3. This seems to be a nondirected and relatively inefficient way of locomotion, with a great expenditure of energy for a small amount of progress.
4. They live in a low (acidic) pH.
5. They would probably not do as well because they are apparently adapted to living in the acidic range.
6. Vinegar eels could be placed in solutions of different pH and their relative rates of growth and multiplication could be measured.
7. The muscles would run the length of the body in continuous bands whose contractions would whip the body from side to side as seen.
8. Yes. The one group seems to have a curled posterior with spines. (These are males.)
9. No.
10. No.
11. The surface is smooth and fairly stiff. The only projections are the spines on the male.
12. The student may not easily distinguish between anterior and posterior,

but there is a definite head end at which the mouth is located, surrounded by three liplike projections. On the male, the posterior end is the curved end with the spines.

13. After examination of a number of reproductive systems, the student should see that there are two types: one reproductive system (the male), joining the rectum at the posterior part of the body; the other (the female), opening through a midventral transverse slit along the middle third of the body. Other differences may be seen between the two systems, but this will be the most obvious.
14. This should suggest that the *Ascaris* is dioecious—that is, existing as separate males and females.
15. No. The female tract opens on the midventral middle third of the body. The male tract opens into the rectum at the posterior end.
16. Those animals in which the reproductive tract opens near the rectum have a curved posterior and prominent spines. These are males.
17. Tenable hypotheses regarding the systems of *Ascaris* would be: the existence of a rudimentary respiratory system and a relatively low oxygen requirement and low rate of metabolism, because of its habitat; a simple digestive tract, because it is living in a medium in which food is abundantly available in a predigested condition; a chemically resistant outer covering to protect against the digestive enzymes of the intestinal tract in which it lives; a simple nervous system with few, if any, sensory organs, because of its relatively sessile, dark mode of life; and an essentially primitive muscular system, because there is no necessity for more than limited locomotion.
18. It is segmented and has a prominent clitellum. Its color may also be a distinguishing characteristic.
19. The vinegar eel whips from side to side by means of longitudinal muscle. The earthworm's locomotion, utilizing both longitudinal and circular muscles, is essentially accordionlike.
20. Openings at the anterior and posterior ends would suggest a mouth and anus and thus, a complete digestive tube.
21. As the ends are different, the animal can be said to have definite anterior and posterior ends.
22. The animal usually moves toward the anterior, or mouth, end.
23. It flows anteriorly, toward the head region, in the main part of the body posterior to the circulatory connectives. Anterior to these, it moves posteriorly, toward them.
24. The rate of contraction will rise perhaps two or three times for each 10° C rise in temperature through a normal biological range. Therefore, the results will be highly variable, depending on temperature.
25. Student's pulse rate will be faster than the contraction of the earthworm's dorsal vessel.
26. A higher rate of metabolism in the human body necessitates a more rapid flow of blood.
27. The student sees no specific sense organs in gross observation.
28. The earthworm is quite sensitive to touch, more so at the head end

than posteriorly; but it reacts to stimuli along the entire length of its body.

29. The earthworm is negatively phototactic.
30. Worms will begin to dig into the loose soil.
31. The anterior end of the body becomes thin and pointed and is forced into the ground, pushing the earth to one side as if plowing. The earthworm will also swallow quantities of earth and pass it through its digestive tract, but this will probably not be noticed.
32. The septa, which occur at the ends of the segments, make the dissection more difficult than that of *Ascaris*.
33. The septa correspond to the rings seen on the outside of the worm.
34. The name Annelida is derived from *annulus*, or ring, and is descriptive of the earthworm.
35. As there seem to be more specialized regions of the earthworm's digestive tract, it is more complex than that of *Ascaris*.
36. The earthworm must process a wider variety of food materials than must *Ascaris*, which lives in a pre-digested food medium and thus has a digestive tract which is adapted to handling such foods.
37. It will be flowing ventrally in the connectives and posteriorly along the ventral blood vessel except for its most anterior portion, where it flows anteriorly.
38. The earthworm's reproductive organs are anteriorly located in the body and those of the *Ascaris* are coiled along the entire length of the body. Those of the earthworm are more compact than the filamentous reproductive tract of *Ascaris*. The earthworm's reproductive organs are irregular spheres and short ducts, while the tract of *Ascaris* is filamentous.
39. No. The earthworm is monoecious and has sex organs of both types in one individual.
40. In the earthworm cross section the cuticle is thinner than in the *Ascaris*. The muscular layer is thicker. The digestive tract is larger and more specialized. Definite excretory organs can be seen in the earthworm, as well as a prominent dorsal and ventral blood vessel and a prominent ventral nerve cord, which differ from anything seen in *Ascaris*.
41. The suspension of the digestive tract in a cavity allows it maximal motility for its peristaltic movements.
42. The nerve cord is ventral.
43. The indentation (typhlosole) provides more surface for absorption of food materials from the digestive tract.
44. The *Ascaris* tract can be simpler because of the fact that it is existing parasitically on predigested foods. The earthworm's tract is specialized to provide maximal absorptive surface for the food obtained from its environment.
45. There is no evidence of a specialized respiratory system, a fact which would lead one to believe that the integument serves as a respiratory structure.

46. The answer to this question can be as extensive as you wish to have it. However, the main idea is that the *Ascaris*, in general, has a simpler group of structures, with the possible exception of its reproductive system, than does the earthworm. This simplicity of structure is correlated with the parasitic mode of existence where many of the life functions of *Ascaris* are performed in part by the host. The earthworm, being a free-living animal, must perform all these functions for itself.

SUGGESTED READINGS

Books

Buchsbaum, R. 1948. *Animals Without Backbones.* University of Chicago Press, Chicago

Chapter 15 discusses roundworms, Chapter 20, earthworms.

Hyman, L. H. 1951. *The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta, the Pseudocoelomate Bilateria.* Vol. 3. McGraw-Hill, New York

Contains a wealth of information on nematode worms.

Stephenson, J. 1930. *The Oligochaeta.* Clarendon Press, Oxford

The most extensive coverage of earthworms extant.

Articles

Eiseley, L. 1956. "Charles Darwin." *Sci. Am.* **194** [2] 62

FILMS

Earthworms, 1957, (sd, c, 11 min), p, Dowling
Earthworm Anatomy, 1961, (sd, b&w or c, 11 min), p or r, Indiana
Worms: *The Annelida*, 1955, (sd, b&w or c, 13 min), p or r, EBF
The Problem of Hookworm Infection, 1954, (sc, sd, 8 min), r, USPH

ANIMALS WITH JOINTED APPENDAGES

EXERCISE 20-2

TIME: One laboratory period.

MATERIALS: See page 165 in the *Student Laboratory Guide.*

This exercise is designed to accompany the portion on the phylum Arthropoda in Chapter 20 of the textbook.

■ **The purpose of this exercise is to study the structure and behavior of two representative arthropods and to compare and contrast the structure and behavior of the arthropods with those of animals previously studied.**

SPECIAL INSTRUCTIONS

Comparisons in this exercise are all external. No dissection is required. The use of the stereoscopic dissecting microscope will aid in locating and observing the various structures under consideration. Since the crayfish and the lobster are so similar, either may be used.

Every effort should be made to have on display live specimens of various stages of grasshoppers in order to demonstrate metamorphosis. The nymphs do not have fully developed wings. The comparison between the young and old grasshoppers will help bring out the idea of molting.

Demonstrations of stages of complete metamorphosis, such as occur in a moth or butterfly, will give additional understanding to the process of metamorphosis. In the parts of this exercise that require close study, the use of preserved specimens is perhaps better, since that eliminates the confusion caused by pinching crayfish and jumping grasshoppers.

TECHNIQUES

If the crayfish are not fed the day before the exercise they may take the small fish offered as food more readily.

Spiracles are difficult to locate on the body of the grasshopper and special attention must be given to pointing them out.

A cotton-plugged test tube makes an admirable container for small, live grasshoppers, and eliminates the problem of trying to examine jumping grasshoppers.

ANSWERS TO QUESTIONS

1. Backward.
2. If the stimulus is strong enough, it moves rapidly backward by flipping the tail forward under the abdomen.
3. Forward or backward.
4. By means of its walking legs.
5. Hard.
6. A greater degree of protection.
7. No, the exoskeleton prevents such motion.
8. At specific joints that are thin spots in the exoskeleton.
9. It protects the animal in the manner of a suit of armor.
10. It is heavy and restricts movement and growth.
11. In the abdomen, this segmentation is obvious; in the cephalothorax, less obvious. If the student understands that there is one pair of appendages per segment, however, the ventral surface gives excellent evidence of complete segmentation.
12. The dorsal surface, particularly the cephalothorax, does not give evidence of regular segmentation from head to tail. However, the ventral surface, with its paired appendages, does.
13. The exoskeleton must be shed periodically to allow the animal to grow

during a brief interval while the new exoskeleton is still soft. Then it becomes rigid through impregnation with mineral salts, especially carbonates.

14. There are 19 pairs of appendages, divided as follows: one pair of antennae; one pair of antennules; six pairs of mouth parts, including mandibles, first and second maxillae, and first to third maxillipeds; one pair of large pincers; four pairs of walking legs; five pairs of swimmerets; and one pair of uropods.
15. No. See the answer to Question 14.
16. The appendages are highly specialized, serving respectively for sensory reception (antennae), food-handling (mouth) parts, offense (pincers), walking (legs), reproduction (first male abdominal appendages), and locomotion (swimmerets and uropods).
17. That the appendages are moving and creating water currents around the animal.
18. The swimmerets and the mouth parts are responsible.
19. Gills are attached to the walking legs and to the second and third maxillipeds.
20. The current of water passes forward over the gills.
21. To a limited extent, because the gills require constant moisture. The crayfish can come on land for short periods where it can live in damp burrows.
22. Eyes, antennae, antennules.
23. At the anterior end of the body.
24. It has paired, jointed appendages; antennae; eyes; mouth parts; and it is segmented.
25. It has only three pairs of legs; only one pair of antennae; is divided into definite head, thorax, and abdomen; lacks gills, pincers, uropods, and swimmerets; and lacks a flexible abdomen used for locomotion.
26. Three, walking, jumping, and flying.
27. Wings, in the adult.
28. Yes.
29. The small stages have proportionately larger heads and thoraxes and lack fully developed wings.
30. The abdomen becomes relatively larger and wings develop.
31. The exoskeleton must be shed.
32. There is no larval or pupal stage in the grasshopper.
33. The larva and the pupa.
34. There is an incomplete metamorphosis because two stages—the larva and pupa—are lacking.
35. Both have eyes and antennae.
36. The grasshopper has both simple and compound eyes and only one pair of antennae. The crayfish has only compound eyes, but two pairs of antennae.
37. No.
38. Some opening or openings through which oxygen could pass into the body and carbon dioxide pass out.

39. A terrestrial environment, with open spaces for jumping and flying.
40. The female has an ovipositor at the tip of the abdomen for depositing eggs. This is lacking in the male.
41. There are separate sexes. The grasshopper is dioecious.
42. The student may have noticed that the first pairs of swimmerets on the male crayfish are modified for passage of sperms to the female. In the female all the swimmerets look alike.
43. Mutation, adaptation, and evolution account for the progressive complexity of the animals studied, from protozoans to arthropods. The student may not express this answer in exactly this fashion, but this is the general idea he should present.

SUGGESTED READINGS

Books

Buchsbaum, R. 1948. *Animals Without Backbones.* University of Chicago Press, Chicago

Chapters 23 through 24 discuss arthropods, including the crayfish and the grasshopper.

Articles

Brown, F. A. 1954. "Biological Clocks and the Fiddler Crab." *Sci. Am.* **190** [4] 34
Hoyle, G. 1958. "The Leap of the Grasshopper." *Sci. Am.* **198** [1] 30
Milne, L. J., and M. J. Milne. 1948. "Insect Vision." *Sci. Am.* **179** [1] 42
Nicholas, Bro. G., F.S.C. 1955. "Life in Caves." *Sci. Am.* **192** [5] 98

FILMS

Crayfish, 1950, (sd, b&w, 11 min), p or r, UW
Crayfish Anatomy, 1961, (sd, b&w or c, 11 min), p or r, Indiana
Crustaceans, 1955, (sd, b&w, or c, 14 min), p or r, EBF
The Grasshopper: A Typical Insect, 1955, (sd, b&w or c, 6 min), p, Coronet
Life Cycle of a Fly, 1951, (sd, b&w, 11 min), p or r, UW
Life Cycle of the Fly, 1960, (sd, b&w, 15 min), p or r, McGraw-Hill
Metamorphosis of Insects, 1961, (sd, c, 10 min), p or r, McGraw-Hill

FORM AND FUNCTION IN THE FROG

EXERCISE 20-3

TIME: Two laboratory periods.

MATERIALS: See page 169 in the *Student Laboratory Guide*.

This exercise is to accompany the section on chordates in Chapter 20 of the textbook.

■ **The purpose of this exercise is to observe the internal structure and function of some of the major organs in the body of the frog. These organs and their functions are essentially the same in all chordates. The study of the organs in the frog's body cavity will contribute to an understanding of human physiology and anatomy.**

SPECIAL INSTRUCTIONS

It is advisable to pith the frogs for student use before class, since instructing individual students in performing this technique is time-consuming. Be sure that the student understands the procedure of pithing, however, and the fact that the frog, even though it appears to be alive, is for practical purposes a dead animal. This will avoid unnecessary complications about the use of live animals for experimental purposes in the classroom.

Pithing is a method of destroying parts of the brain and spinal cord. This method of treatment is preferable to use of an anesthetic and is easier and more reliable. The animal requires no special attention in the experiment and may be discarded at the conclusion of the exercise without further care. In single pithing, only the brain is destroyed; in double pithing both the brain and the spinal cord are destroyed.

TECHNIQUES

To pith the frog, remove it from its container, and hold it firmly by the hind legs. Wash it with water and hold it in the left hand with its head exposed. Place the head between the index and middle fingers with the thumb on the frog's back (see Figure 20-3-1). Bend the head forward at a right angle to the body. Either locate the base of the skull with the thumbnail of the right hand, or place the needle on the head, midway between the eyes, and applying pressure, draw the needle posteriorly toward the body until it enters the groove formed by the junction of the skull and spinal cord. Apply pressure and direct the needle forward through the skin over the groove. Push the needle forward into the brain cavity using a twisting, side-to-side movement. A few attempts may be necessary at the beginning to locate the exact spot. As the brain is destroyed, the frog will become limp, an effect of shock, and will remain so for about five minutes. When the shock has worn off, the frog's leg will contract in response to pinching the skin. Test the eyelid reflex by gently touching the cornea of the eye. If the eyelid shows no reflex—that is, no raising of the lower lid of either eye—the destruction of the brain is complete. If the eye shows a reflex, by raising the lower lid, the pithing is not complete and must be repeated.

To double pith the animal, withdraw all but the tip of the needle from the brain cavity after destroying the brain. Change the direction of the needle toward the hind legs and parallel to the spinal cord. Find the soft center of the spinal column by gently probing with the needle, then thrust the needle through the core toward the hind legs. When the needle is

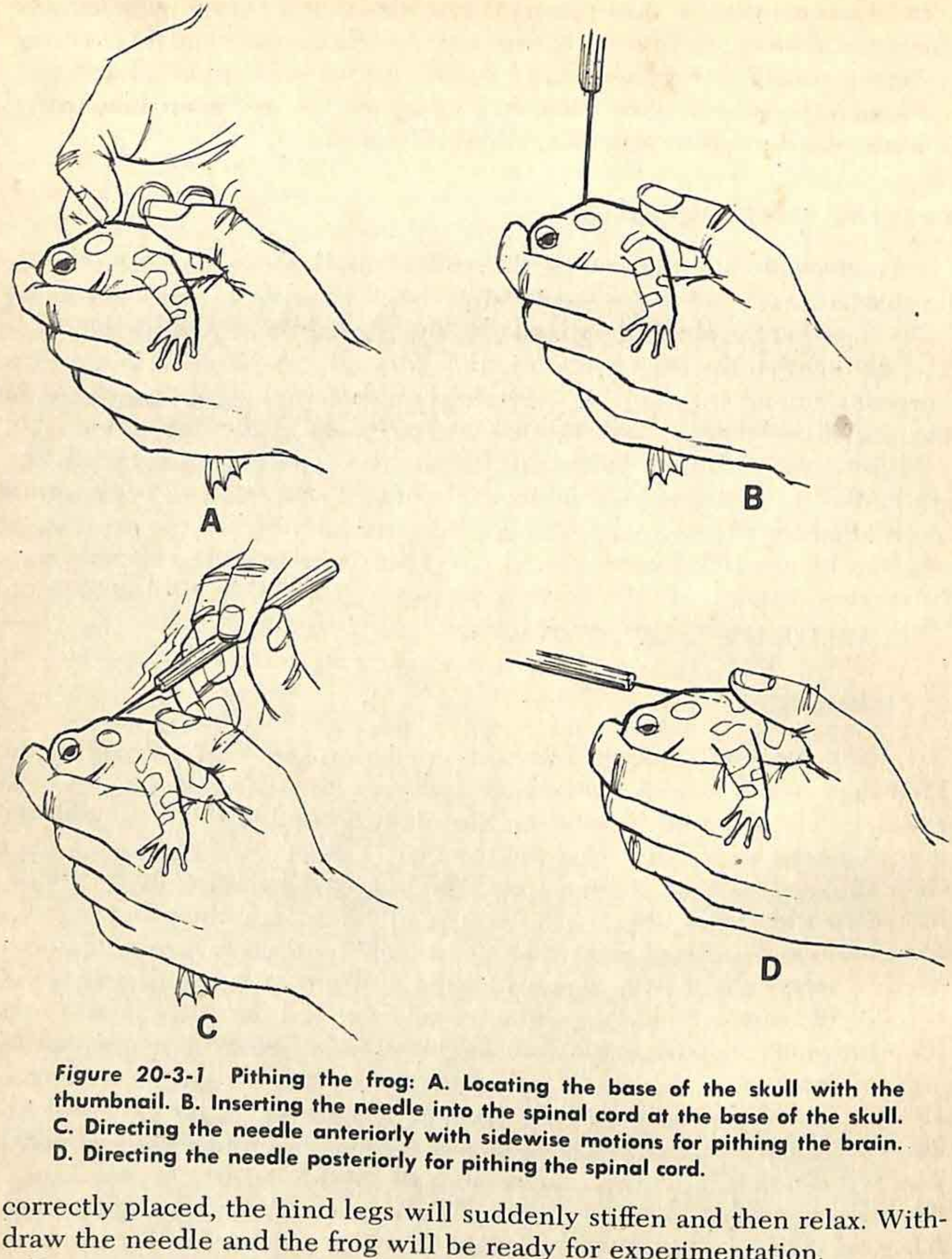


Figure 20-3-1 **Pithing the frog: A. Locating the base of the skull with the thumbnail. B. Inserting the needle into the spinal cord at the base of the skull. C. Directing the needle anteriorly with sidewise motions for pithing the brain. D. Directing the needle posteriorly for pithing the spinal cord.**

correctly placed, the hind legs will suddenly stiffen and then relax. Withdraw the needle and the frog will be ready for experimentation.

Be certain to caution the students to make shallow incisions through the skin and muscles so that underlying organs will not be damaged.

Urethane can be substituted for salt solution to induce peristalsis in the intestine.

Concentrated instant coffee solution can be used instead of caffeine.

Adrenaline chloride is a common ingredient in some nose drops. Commercial preparations such as No-Doz have been used instead of adrenaline, but are not as successful for this purpose.

For best results, be sure the frogs are stored in a refrigerator between exercises. However, if a refrigerator is not available, they can be preserved in 50% alcohol or 5% formalin.

Caution the student about inflating the lungs. The tendency is to use too much air, and the lungs then burst.

ANSWERS TO QUESTIONS

1. It means that death does not result in the cessation of all living processes simultaneously. For example, certain organs and processes continue to operate autonomously despite the fact that the animal is, to all intents and purposes, dead.
2. Life is apparently an intrinsic property of the individual parts of an animal, and even of individual cells. It is not simply an all-or-none phenomenon of the whole animal.
3. The skin is very loosely bound to the underlying muscles and can be easily removed.
4. No, but it indicates that the heart is what we would call alive.
5. Variable.
6. Slower.
7. Three.
8. Yes.
9. No.
10. From the atria to the ventricle.
11. Blood with a low oxygen supply.
12. By definition, in an artery. All vessels that carry blood from the heart are called arteries.
13. Blood with a good oxygen supply.
14. In an artery.
15. The heartbeat should slow down.
16. The heartbeat should accelerate.
17. The heartbeat should accelerate.
18. Cold water is an inhibitor; warm water and either caffeine or adrenaline are accelerators.
19. Its large amount of thin-walled, moist surfaces.
20. The lungs are relatively small.
21. No, the lungs are in the same cavity as the other viscera.
22. Peristalsis should increase.
23. Posteriorly.
24. They move food along the digestive tract.
25. The intestine seems to quiver or shake.
26. Calcium seems to prevent shaking or quivering of muscle.
27. Certain parts are still viable and functioning long after the animal has been considered dead.
28. Three.
29. The identification of the sides would vary, depending upon whether the individual doing the dissection was at the head or the foot of the

animal, and thus the directions would change as the viewer changed his position.

30. The valve holds the food in the stomach until the preliminary breakdown in the acidic medium is complete. It then opens and lets the food into the intestine, where the pH is alkaline. If there were no valve, food would constantly be passing through and leaving little chance for the stomach to do its preliminary work.
31. It was attached by means of a membrane to the dorsal body wall.
32. Yes. This membrane extends along the entire length of the digestive tract.
33. There is no structural indication that the spleen is a part of the digestive system.
34. It is larger in diameter than the small intestine and is also in the general position of the large intestine found in the human being.
35. Variable, depending on student observation, but approximately 15 cm long.
36. Variable, depending on the frog used, but 3 to 4 cm long is usual.
37. Variable, depending on the frog used, but approximately 5 mm.
38. Variable, depending on the animal used, but approximately 10 mm.
39. There is no cloaca in the human being.
40. That a basis exists in each individual, regardless of its sex, for developing both the male and female systems.
41. The egg passes from the ovary to the body cavity. It then enters the oviduct, and passes through the uterus and cloaca to the exterior.
42. Sperms pass from the testes through tiny sperm ducts leading into the kidneys. From the kidneys they travel through the excretory tubules (ureters) to the cloaca and thence to the outside.
43. Urine proceeds from the kidney through the ureter to the cloaca. It is stored in the bladder which opens off the cloaca, until it passes to the outside, through the cloaca again.
44. This will depend on whether the frog had been fed before it was killed.
45. The food will be small fish, insects, or similar animal food.
46. A carnivore.
47. The inside lining of the stomach is covered with ridges that make it rough.
48. The internal surface of the intestine is covered with small projections, the villi.
49. It differs from the inside of the stomach in lacking the heavy ridges.
50. The numerous small projections increase the amount of surface of the small intestine available for absorption of digested food.
51. The answers will vary, depending on how thoroughly the subject has been covered. However, the student should indicate an understanding of the fact that the internal organs of the frog and their functions are similar to the internal organs and their functions in the human body. There are differences in detail, but in general the systems and organs are similar enough to allow comparisons to be profitably made between these two chordates.

SUGGESTED READINGS

Books

Oliver, J. 1955. *Natural History of North American Amphibians and Reptiles.* Van Nostrand, Princeton

Articles

Gerard, R. W., 1948. "The Dynamics of Inhibition." *Sci. Am.* **179** [3] 44
Potter, R. K., 1950. "Frog Calls." *Sci. Am.* **182** [5] 46
Sperry, R. W. 1956. "The Eye and the Brain." *Sci. Am.* **194** [5] 48

FILMS

Life Cycle of the Frog, 1950, (sd, b&w, 11 min), p or r, UW
The Frog, 1957, (sd, b&w or c, 11 min), p or r, EBF
Frog Anatomy, 1959, (sd, b&w or c, 17 min), p or r, Indiana
Frogs and Toads, 1956, (sd, b&w or c, 11 min), p or r, McGraw-Hill
Amphibians, 1957, (sd, b&w or c, 11 min), p, Coronet

ANIMAL CLASSIFICATION

EXERCISE 20-4

TIME: One laboratory period.

MATERIALS: See page 173 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on animal classification in Chapter 20 of the textbook.

■ **The purpose of this exercise is to familiarize the student with methods of classifying animals and of identifying animals whose names are already in existence. From this exercise students should gain familiarity with the great diversity within the animal kingdom and come to appreciate some of the relationships between various groups of animals.**

SPECIAL INSTRUCTIONS

It may be possible for the students to collect representatives of various phyla and classes for keying out in this exercise. This opportunity to involve the students should not be lost.

Keep in mind that this is not an exercise in *naming* but rather an exercise in *how* animals are classified. Earlier, the students have used a slightly different kind of key in classifying plants. Thus the idea is not completely foreign to them. However, their knowledge of terminology may have to be reinforced even though it has been held to a minimum in these keys.

The students should be told that the more complete the key, the better the job it will do. They should be constantly reminded that the keys in this exercise are deliberately simple in order to demonstrate systematic principles and not to exemplify detailed systematic procedures. Many animals cannot be keyed by these keys, but students will come to realize that, in order to key out all the animals in a group, the keys, of necessity, have to be complex.

In addition to keying animals by phylum and class, an opportunity is given for the student to construct his own key to the classes of mollusks. This is particularly helpful if representatives of the molluscan classes are available for demonstration and use.

TECHNIQUES

This exercise is designed to be accomplished in one period. More periods than one can be used but to do so will place a greater emphasis on the mechanics of systematics than is deemed desirable.

Additional animals and other keys to various animals can be used to augment this exercise.

Students can participate individually or in groups. While the exercise is based on preserved specimens in the school collection, every effort should be made to supplement them with specimens collected by the students after school. The variety of collectable organisms in the community — in homes and outdoors, or to be seen in museums or at taxidermists, and so forth — is great. The more comprehensive the collection becomes, the broader the over-view of the animal kingdom will be to the student.

If the students are asked to collect, collecting courtesy should be emphasized. Necessary adaptations to local situations should be made. If park permits are necessary, arrangements for these should be made well in advance. Students should realize that they are trespassing whenever they enter private property without permission. This exercise may offer an opportunity for a bit of citizenship education.

Because the physical arrangements of facilities in schools vary so widely it is not possible to set up a uniform set of procedures for the laboratory study of animals. Each teacher will arrange the laboratory directions in such a way as to get the materials to the student in the simplest manner. Directions may be put on the blackboard or on mimeographed sheets.

This exercise is designed as an illustrative study and not an experiment. Students should, in using the keys, come to see that animals fall into related groups but that the various characteristics are not of equal value in classifying animals and that structural features have more significance than do habitats.

ANSWERS TO QUESTIONS

1. The greater detail in Key B allows for a slight refinement of keying technique over that possible with Key A.

2. All the classes within a phylum bear the characteristics common to the phylum.
3. One would expect the classes within a phylum to be more similar to one another than they are to classes belonging to a different phylum.
4. Because all members of the phylum would have certain features in common that might not be shared with members of another phylum.
5. Superficial morphological characters.
6. Yes.
7. It would be very long and highly detailed.
8. No, because all insects would have an exoskeleton and this would not serve to differentiate between them.
9. No, because all insects would have six legs and this would not be a differentiating characteristic.
10. It is possible to separate groups of insects as to whether or not they have wings.
11. The differences in bodily form will undoubtedly be the major feature of differentiation between various groups of insects.
12. Yes. It is possible to separate animals on the basis of their habitat, but the separations will not be as fine as those designed to separate them morphologically.
13. Animals may be separated to some extent on the basis of geographical distribution.
14. Food habits serve to identify certain groups of animals—herbivores and carnivores, for example.
15. Economic importance, because it is a man-determined characteristic, could not serve as a natural key characteristic in separating animals.
16. Because the color will not be a broadly distinguishing characteristic. For example, one could have brown insects, birds, mammals, fishes, and shells, which would not belong even to the same phylum. But one can distinguish two very similar species of butterflies on the basis of their color.
17. Despite the fact that they all have wings, the wings are only analogous and insects, birds, and mammals would not fit into the same categories based on other characteristics.
18. Because they have a number of homologous traits in common, including food habits and basic structural similarities.

SUGGESTED READINGS

Books

Abbott, R. T. 1954. *American Sea Shells*. Van Nostrand, Princeton
Axelrod, H., and L. Schultz. 1955. *Handbook of Tropical Aquarium Fish*. McGraw-Hill, New York
_____ and W. Vorderwinkler, 1956. *Salt-Water Aquarium Fish*. Sterling, New York
Burt, W., and R. Grossenheider. 1952. *A Field Guide to the Mammals*. Houghton Mifflin, Boston
Hanson, E. D. 1961. *Animal Diversity*. Prentice-Hall, Englewood Cliffs, N.J.
Jaques, H. E. 1947. *How to Know the Insects*. W. C. Brown, Dubuque, Iowa

Netting, G., and G. Orton. 1950. *A Field Guide to the Amphibia and Reptiles.* Houghton Mifflin, Boston

Palmer, R. 1954. *The Mammal Guide.* Garden City, New York

Peterson, R. 1948. *How to Know the Birds.* Houghton Mifflin, Boston

Pettingill, O. 1951. *A Guide to Bird-finding East of the Mississippi.* Oxford Univ. Press, New York

______. 1951. *A Guide to Bird-finding West of the Mississippi.* Oxford Univ. Press, New York

Swain, R. 1948. *The Insect Guide.* Doubleday, New York

FILMS

The Multicellular Animal, Part IV: *Form and Function*, No. 1, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Mollusks: *Snails, Mussels, Oysters, Octopuses, and their Relatives*, 1955, (sd, b&w or c, 14 min), p or r, EBF

Insect Collecting, 1960, (sd, c, 14 min), p, Dowling

Insect Mounting and Preserving, 1961, (sd, c, 14 min), p, Dowling

Diversity of Animals, Part VIII: *Nature of Diversity*, No. 1, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

  Order in Diversity, No. 2, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

  Crustacea and Their Allies, No. 5, 1962, (sd, b&w or c, 28 min), p or r, AIBS (McGraw-Hill)

  Fishes, Amphibians and Reptiles, No. 7, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

  Insects and Spiders, No. 8, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

  Mollusks: A Story of Adaptation, No. 9, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

  Birds and Mammals, No. 10, 1962, (sd, b&w or c, 28 min), p or r, AIBS(McGraw-Hill)

DIGESTION OF FOODSTUFFS

EXERCISE 21-1

TIME: One laboratory period.

MATERIALS: See page 177 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on the digestion of food in Chapter 21 of the textbook.

■ **The purpose of this exercise is to classify some common foodstuffs by their starch, sugar, fat, or protein content and to correlate their chemical composition with the locations where they will be broken down in the human digestive tract.**

SPECIAL INSTRUCTIONS

This exercise should be introduced by the explanation that a food, such as a slice of bread, a glass of milk, or a piece of meat, is not a single substance. Each will be made of a complex mixture of compounds. Compounds which nourish the body are called nutrients. They have been classified by biologists as starches, sugars, fats, proteins, vitamins, or minerals.

The student should be able to develop the concept that in a food such as a carrot or banana, the various nutrients are so intermixed that no one of them can be recognized except by chemical tests. For example, no matter how thoroughly starch is mixed with other nutrients in a banana, a drop of iodine, a starch detector, will turn blue-black when placed on a piece of banana. No matter how thoroughly sugar may be mixed in a glass of milk, the sugar can be detected with Benedict's solution.

In this exercise, students are given the opportunity to use some chemical tests to discover the nutrients present in foods. The nutrients to be tested for are starches, sugars, fats, and proteins. The tests for vitamins and minerals are too difficult and time-consuming to be done during a single laboratory period.

The students should already be familiar with the test for starch and the test for sugars. However, they have not encountered the biuret test for protein before, and this should be demonstrated so that students will understand the reaction to the presence of protein.

TECHNIQUES

The class may be organized in a variety of ways for performing this exercise. The method you select may be determined by the availability of time and equipment. All students can test all foods and all unknowns.

The Unknowns listed under Materials in the *Student Laboratory Guide* should be prepared beforehand and labeled. The test results can be written on the blackboard. You, however, should have a key to the unknowns. In place of Benedict's solution, Fehling's solutions A and B can be used, or glucose test paper or Clinitest tablets, available from the Ames Co., Inc., Elkhart, Ind., can be used. The advantage of the papers and the tablets is that heating is not required and the test is thus somewhat simplified.

The fat, trimmed from meat, should be cut into small enough pieces to place in test tubes before class. The cutting of fat in the classroom is sometimes very messy. If fat is handled with forceps instead of fingers, greasy fingerprints are also avoided.

Probably the simplest way of preparing unknowns is to purchase assorted strained baby foods. The labels can be easily removed and their appearance fairly well masked by the addition of food coloring or dyes, since they possess a nearly uniform consistency. If desired, two of the foods may be mixed. Some suggestions are strained beef, strained carrots, strained green beans, and strained apricots.

The same test tubes can be used over again in the tests by rinsing them thoroughly between each test.

ANSWERS TO QUESTIONS

1. A striking color change will take place in Tube 1, since it was a starch solution.
2. Starches.
3. In the presence of sugar, a yellow or reddish precipitate of cuprous oxide forms. There should be a pronounced reaction in Tube 2, the one containing glucose solution.
4. Glucose, and other (reducing) sugars.
5. Peptones or proteoses give a pink reaction in this test. The most pronounced reaction will be noticed in Tube 3, the one containing egg albumen.
6. Proteins broken down to proteoses and polypeptides.
7. A control.
8. The piece of fat from meat.
9. The student may be led to the erroneous conclusion that certain foods contain only one type of nutrient, depending on the food used. For example, a positive starch test in a banana may indicate to the student that it contains only starch. However, with the usual foodstuffs, he should get positive reactions for starches, proteins, fats, and sugars.
10. The student may find that some foods yield a positive test for carbohydrates, fats, and proteins.
11. Fats seem to be absent, but can be detected with finer techniques using ether as a solvent.
12. Whole milk contains a greater proportion of fat, as well as protein and carbohydrate.
13. This will depend upon the food used, but the student will assign digestion of proteins to the stomach and intestine; starches to the mouth and intestine; and fats to the intestine.
14. Proteins and fats would be least affected by not chewing thoroughly, because only starch digestion commences in the mouth.
15. Yes, because they would be digested in the intestine.
16. Yes, their digestion continues in the intestine.
17. Fats.
18. Those thoroughly chewed. Reactions take place at surfaces. Chewing food into small pieces gives greater surfaces on which enzymes can act and therefore digestion will be most effective.
19. Proteins.

SUGGESTED READINGS

Books

Best, C. H., and N. Taylor. 1955. *The Physiological Basis of Medical Practice.* Williams and Wilkins, Baltimore

Fulton, J. F. 1955. *Textbook of Physiology.* W. B. Saunders, Philadelphia

Articles

Doty, P. 1957. "Proteins." *Sci. Am.* **197** [3] 173

Fieser, L. F. 1955. "Steroids." *Sci. Am.* **192** [1] 52

Fruton, J. S. 1950. "Proteins." *Sci. Am.* **182** [6] 32
Linderstrom-Lang, K. U. 1953. "How Is a Protein Made?" *Sci. Am.* **189** [3] 100
Stein, W. H., and S. Moore. 1961. "The Chemical Structure of Proteins." *Sci. Am.* **204** [2] 81
Trowell, H. C. 1954. "Kwashiorkor." *Sci. Am.* **191** [6] 46
Williams, R. J. 1948. "Alcoholics and Metabolism." *Sci. Am.* **179** [6] 50

FILMS

Alimentary Tract, 1938, (sd, b&w, 11 min), p or r, EBF
Digestion of Foods, 1938, (sd, b&w, 11 min), p or r, EBF
Digestion (Mechanical), 1939, (sd, b&w or c, 17 min), p or r, UW
Digestion (Chemical), 1949, (sd, b&w or c, 19 min), p or r, UW
Human Body: *Digestive System*, 1958, (sd, b&w or c, 13½ min), p, Coronet
Human Digestion, 1961, (sd, b&w, 10 min), p or r, Contemporary
The Multicellular Animal, Part IV: *Ingestion and Digestion*, No. 3, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)
The Multicellular Animal, Part IV: *Chemical Machinery*, No. 5, 1962, (sd, c, 31 min), p or r, AIBS(McGraw-Hill)

THE LIVING INVERTEBRATE HEART

EXERCISE 22-1

TIME: One laboratory period.

MATERIALS: See page 179 in the *Student Laboratory Guide.*

This exercise is to accompany the section on the heart in Chapter 22 of the textbook.

■ The purpose of this exercise is to have the students observe the living invertebrate heart and through their own observations and experiments to determine the rate of its beat and the effects of changes in the environment and the effects of drugs on the rate of heartbeat.

SPECIAL INSTRUCTIONS

The normal heartbeat of *Daphnia* ranges from 300 to 350 beats per minute. Because this rate is more rapid than a student can normally be expected to count, an approximate rate of beat can be obtained by tapping with a pencil point on paper in rhythm with the heartbeat. The taps must be in a continuous line back and forth as shown in Figure 22-1-1. At the

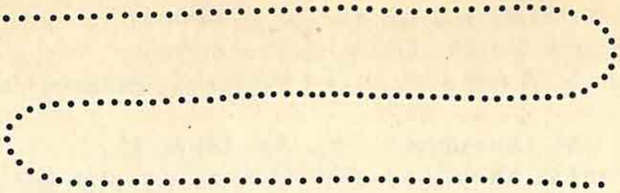


Figure 22-1-1 Heartbeat record of *Daphnia*.

conclusion of the observation period students can count the number of dots, and thereby obtain the rate of heartbeat. This procedure must be explained to the students before they start to count the heartbeats in Part A.

By observing the effects of temperature, tranquilizers, alcohol, and other drugs on the rate of heartbeat, students will gain an understanding of the use of invertebrates such as *Daphnia* in testing concentrations of drugs and solutions of unknown concentration.

TECHNIQUES

Daphnia may be collected from ponds and lakes during most of the months of the year, and can also be ordered from biological supply houses. Often they can be obtained at aquarium supply stores or fish hatcheries.

There are several species of *Daphnia. Daphnia magna* is the largest and therefore the preferable species for use. *Daphnia pulex* and *Daphnia longispina* are only about half the size of *Daphnia magna* but will prove satisfactory for the purposes of this exercise.

If *Daphnia* are cultured, the culture can be maintained for one or two weeks in pond water kept out of direct sunlight. If the culture is to be maintained for a longer period, a culture medium must be provided. Perhaps one of the simplest methods of culture involves placing *Daphnia* in tap water which has stood for several hours. The *Daphnia* can be fed a suspension of yeast in water—enough suspension added to make the water milky. When the water has cleared, add more yeast suspension. The water should be changed once a month. An aerator may be used or the culture may be stirred once a day.

Many types of culture media are suggested in the literature for *Daphnia*, particularly in Lutz, *et al.* 1959. *Culture Methods for Invertebrate Animals.* Dover, New York.

Tubifex worms and mosquito larvae can be used in lieu of *Daphnia*.

You may prefer to put the chemical solutions to be used in containers labeled A, B, C, D, etc., and not tell the students what these are until later. This will insure that the results will not have been deduced from previous knowledge of the effects of these chemicals.

The low power of the compound microscope may be used, but the binocular dissecting microscope is preferred.

For the data, class averages are more reliable than the data from a single team. Data from the results on a single organism may vary considerably.

The control for these experiments is to determine the normal rate of heartbeat each time before adding another solution.

The stimulants and tranquilizers called for can be secured by prescription from your local doctor. Five-mg tablets can be crushed with a mortar and pestle and dissolved in 25 ml of water. Better results may be obtained if this solution stands for about an hour to allow the filler material to settle. Some teachers prefer to filter. Should the solutions be too strong and kill the *Daphnia*, they may be further diluted.

In one school where this experiment was performed, 75 mg of chlorpromazine were dissolved in 50 ml of water, and one tablet of dexedrine sulfate was dissolved in 15 ml of water. In addition, 45% alcohol and plain cola were tested. Class averages showed heartbeat rates as follows: chlorpromazine, 98; dexedrine sulfate, 272; alcohol, 3; cola, 238; normal, 236.

ANSWERS TO QUESTIONS

1. 2. and 3. It is not expected that all members of the class will record a similar rate of heartbeat either at room temperature, at lower temperatures, or at higher temperatures.
4. The differences can be accounted for by the variability of living materials (variation among the individual *Daphnia* used), uncontrolled environmental conditions (temperature, pH, light, etc.), and variation in accuracy of student observations.
5. The graphs should indicate lower temperatures reduce heartbeat rate.
6. There is not an equal difference for each 5° drop in temperature. If the drops in temperature were equal, the graph would resemble the dotted line in Figure 22-1-2 following. The actual graph should approximate more the solid line in the figure.

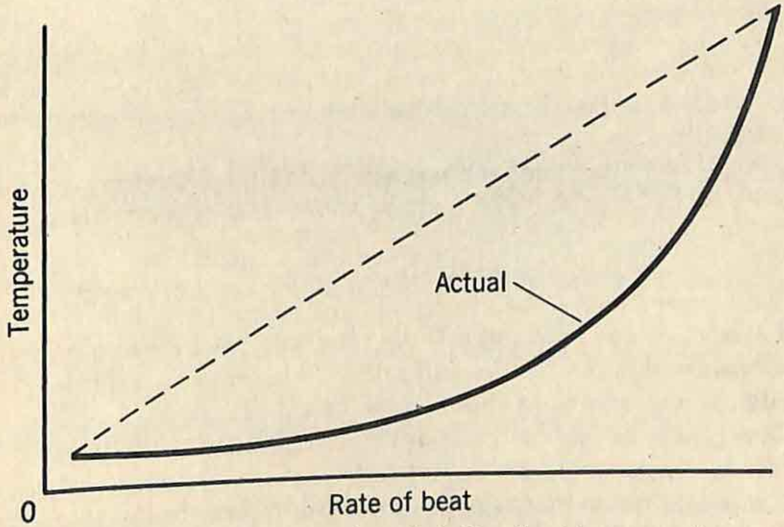


Figure 22-1-2 **Relationship of *Daphnia* heartbeat to temperature.**

7. The higher the temperature, the greater the increase in rate of heartbeat.
8. The chlorpromazine should reduce the heartbeat, depending on the concentration used.
9. This question obviously cannot be answered from the experimental data, for the student does not use *all* tranquilizers. He might speculate that they would all have the same effect, but this experiment does not verify this.
10. The dexedrine sulfate solution increases the rate of heartbeat, depending upon the concentration used, to 270 or more beats.
11. Alcohol reduces the heart rate, but the amount depends upon the concentration used. Of all the chemicals used, it will probably produce the most drastic reduction.
12. Once its reaction to known concentrations of a drug has been plotted, *Daphnia* could be used as a test organism to assay the concentration of some unknown solution of the drug.

SUGGESTED READINGS

Books

Edmondson, W. T. (ed.) 1959. *Fresh-water Biology.* Wiley, New York
Chapter 27 discusses *Cladocera.*

Garnett, W. J. 1953. *Fresh-water Microscopy.* Dover, New York

Kabat, E. 1956. *Blood-group Substances: Their Chemistry and Immunochemistry.* Academic Press, New York

Articles

Anon. 1951. "Heart Muscle." *Sci. Am.* **185** [2] 48
Burnet, M. 1961. "The Mechanisms of Immunity." *Sci. Am.* **204** [1] 58
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Zucker, M. B. 1961. "Blood Platelets." *Sci. Am.* **204** [2] 58

FILMS

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Body Defenses Against Disease, 1937, (sd, b&w, 11 min), p or r, EBF
Body Fights Bacteria, 1948, (sd, b&w, 17 min), p or r, McGraw-Hill
Frog Heartbeat, 1960, (sd, c, 7 min), p or r, Thorne
Heart and Circulation, 1937, (sd, b&w, 11 min), p or r, EBF
How Our Bodies Fight Disease, 1955, (sd, b&w, 8 min), p or r, EBF

CAPILLARY CIRCULATION

EXERCISE

22-2

TIME: One laboratory period.

MATERIALS: See page 183 in the *Student Laboratory Guide.*

This exercise is to accompany the section on capillary circulation in Chapter 22 of the textbook.

■ **The purpose of this exercise is to allow the student to see circulation in the capillaries and to understand the function of the capillary network as that part of the circulatory system where exchanges of materials occur between the blood and the cells of the body.**

SPECIAL INSTRUCTIONS

In order to reduce possible confusion in handling the live goldfish, it may be desirable for you to demonstrate to the class the proper technique.

"Invitations to Enquiry," No. 36 in the *Biology Teachers' Handbook* provides additional information on the experimental evidence of function through a discussion of the valves of the veins.

TECHNIQUES

Goldfish can be anesthetized if desired, without permanent harm, with MS-222 (Sandoz Pharmaceutical Division, Sandoz Inc., Hanover, N.J.). It is to be used in a concentration of about 1 : 3500 for a 5-cm to 8-cm goldfish. The exposure should be for 4–6 minutes at room temperature.

It is important that the gills are covered with wet cotton while the circulation is being observed, and that the tail is moistened occasionally.

The measurements called for can be made using the techniques presented in Exercise 3-2.

The half-slide on top of the tail may not be necessary if the fish are unusually quiet.

If desired, guppies, minnows, or other suitable fish may be substituted for goldfish. Facial tissue can be substituted for cotton.

If the frog is used instead of the goldfish, 1 ml of 10% urethane can be used to anesthetize the frog. It should be tied to a board with a hole in it over which the webbing of the hind foot can be placed.

Circulation can be similarly observed in the tails of tadpoles.

ANSWERS TO QUESTIONS

1. and 2. Depend upon student observation.
3. The sum of the diameters of the capillaries is greater than the diameter of the arteriole from which they arise.

4. The surface-to-volume ratio is greatest in the capillaries.
5. Total cross-sectional area through which blood can pass is greater in the capillaries than in the arteriole.
6. The blood flows more slowly through the capillaries because of their greater surface-to-volume ratio and their greater cross-sectional diameter.
7. Frequently students describe the blood flow in capillaries as more rapid than in the arterioles, even though measurement of the time required for a blood cell to pass through a capillary contrasted with the time necessary for it to pass through an equal distance of an arteriole will show this conclusion to be erroneous.
8. The shape of the red blood corpuscle in the fish is oval.
9. They pass through the capillaries in single file.
10. Since the red blood cells pass through the capillaries in single file, they present a maximum surface for diffusion through the capillary wall. If they were to pass through in clumps, the surfaces of the inner blood cells would not be in contact or close to the capillary wall.
11. Depends upon student observation and measurement.
12. The blood will move less rapidly than in an individual capillary because of reduced pressure.
13. The venules are larger in diameter than the arterioles.
14. The thinner the wall, the more rapid the diffusion of substances in and out of the bloodstream.
15. You would not expect an exchange between red blood cells and body cells across arteries or veins because of the thick walls of these vessels.
16. No, because of the lower metabolic rate and lessened activity of the web of the fish's tail in contrast to muscle, for example.
17. You might assume that capillaries would be closer together in muscle than in the tail web. because of the greater metabolic rate in muscle.
18. The general red color of vertebrate meat indicates an excellent blood supply.

SUGGESTED READINGS

Books

Prosser, C. L. D., W. Bishop, F. A. Brown, Jr., T. L. Jahn, and V. J. Wulff. 1960. *Comparative Animal Physiology.* W. B. Saunders, Philadelphia

Articles

Ponder, E. 1957. "The Red Blood Cell." *Sci. Am.* **196** [1] 95

FILMS

The Blood, 1960, (sd, b&w or c, 16 min), r or p, EBF

Subcutaneous Blood Flow in the Bat: *Active Vasomotion*, 1948, (sd, b&w, 11 min), r, Indiana

Subcutaneous Blood Flow in the Bat: *Behavior of Blood and Vascular Components*, 1948, (sd, b&w, 11 min), r, Indiana

Work of the Blood, 1957, (sd, b&w or c, 13 min), p or r, EBF

HUMAN RESPIRATION

EXERCISE

23-1

TIME: One laboratory period.

MATERIALS: See page 185 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on respiration in man in Chapter 23 of the textbook.

■ **The purpose of this exercise is to determine the amount of carbon dioxide produced by the student metabolisms and thus to obtain an indication of the variability in respiration rates of the human body.**

SPECIAL INSTRUCTIONS

You may wish to have students review the chemistry in the earlier chapters in the textbook in order to refresh their minds about atoms and molecules.

The student is asked in this exercise to take for granted a certain amount of chemical information given in the introduction. You may wish to expand upon this material. However, for the purposes of this exercise it is not necessary for the student to understand more than the concept of the micromole and the use of an indicator.

It is suggested that you use "Invitations to Enquiry," No. 26, in the *Biology Teachers' Handbook* in conjunction with this exercise. This invitation deals with the quantitative aspects of linear relationships relative to oxygen and carbon dioxide in respiration.

TECHNIQUES

You may wish to acquaint the students with the chemical reactions which he will see demonstrated in this exercise. For example, $CO_2 + H_2O \rightarrow H_2CO_3$ (which is the same carbonic acid occurring in soft drinks and always in the bloodstream). NaOH (caustic soda or lye) + $H_2CO_3 \rightarrow$ $NaHCO_3$ (which is sodium bicarbonate or baking soda, occurring as a buffer in the blood) + H_2O. You may wish to explain the phenomenon of buffering at this time by pointing out that sodium bicarbonate serves as a buffer, that is, a protection to some extent against pH changes. Normally sodium bicarbonate is more or less completely ionized into sodium ions and bicarbonate ions. If a little hydrochloric acid is added, you would expect the solution to become much more acidic. Actually, however, there is a rather slight pH change, because the hydrogen ions from the hydrochloric acid have a chance to react with the bicarbonate ions to form carbonic acid, which is a weak acid (comparatively few of its molecules ionized). In effect, the free hydrogen ions of the added hydrochloric acid are being taken out of circulation, and consequently the hydrochloric acid will not change the pH appreciably.

While it is generally assumed that the water used in this experiment will be of pH 7 (neutral) this is not always the case. The pH of the water should first be determined and then made slightly alkaline with NaOH so that a pink color appears when phenolphthalein is added.

Caution the students using the sodium hydroxide solution to avoid contact with hands and clothing. The mixture should be swirled gently so that it will not splash.

Caution the students to add only a drop of NaOH at a time to the acidified solution. There is a tendency to add the NaOH too rapidly and to end up with too much NaOH.

ANSWERS TO QUESTIONS

1. If the water is neutral or slightly alkaline, the phenolphthalein should turn pink.
2. If the phenolphthalein solution turns pink, the solution is alkaline.
3. The solution should become colorless.
4. The pH is changing from alkaline to acidic.
5. Carbonic acid.
6. Depends on student data.
7. No. The results are likely to be variable.
8. Depends on class data.
9. Variation indicates a difference in metabolic rate which may result from differences in activity, physical condition, sex, or any other variable that alters the rate of cellular respiration.
10. It is an indicator of the amount of cellular respiration occurring. The more carbon dioxide produced, the greater the rate of oxidation of glucose and other fuels.
11. Variation in metabolism among the members of the class depends on a number of variables, including activity, physical condition, and sex. (The rate of basal metabolism is lower in girls than in boys.)
12. More carbon dioxide should be produced by the active individual than by the resting one.
13. If the hypothesis is based on differences in physical activity, this should indicate a relationship between amount of activity and carbon dioxide production.
14. Yes. There is a relationship between the amount of oxygen consumed and the amount of carbon dioxide produced.

SUGGESTED READINGS

Books

Krogh, A. 1959. *A Comparative Physiology of Respiratory Mechanisms.* University of Pennsylvania Press, Philadelphia

Articles
Adrian, E. D. 1950. "Physiology." *Sci. Am.* **183** [3] 71
Clements, J. A. 1962. "Surface Tension in the Lungs." *Sci. Am.* **207** [6] 120
Deevey, E. S., Jr. 1951. "Life in the Depths of a Pond." *Sci Am.* **185** [4] 68
Drinker, C. K. 1949. "The Physiology of Whales." *Sci. Am.* **181** [1] 52
Gray, G. W. 1955. "Life at High Altitudes." *Sci. Am.* **193** [6] 59
Williams, C. M. 1953. "Insect Breathing." *Sci. Am.* **188** [2] 28

FILMS

Cell Biology, Part I: *Cell Respiration*, No. 5, 1960, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)
The Multicellular Animal, Part IV: *Respiration*, No. 4, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)
Mechanisms of Breathing, 1954, (sd, b&w, 11 min), p or r, EBF

HUMAN KIDNEY FUNCTION

EXERCISE
24-1

TIME: One laboratory period. (Student diets must begin the day before the exercise.)

MATERIALS: See page 187 in the *Student Laboratory Guide*.

This exercise is designed to accompany the section on the excretory system in Chapter 24 of the textbook.

■ **The purpose of this exercise is to demonstrate the role of the kidney in excretion and in maintaining homeostasis within the blood.**

SPECIAL INSTRUCTIONS

This exercise should be strictly on a voluntary basis. To avoid identification of specific urine samples, letters may be used on the sample bottles and all the samples from a particular diet may be pooled. Enough urine is usually available from volunteers so that all students can run the tests. Since very small quantities are used for each test, state the amount of urine you wish each volunteer to bring to class, based on the number of volunteers.

The student volunteers should be alerted at least a week before the exercise is to be done so that they will understand the necessity for rigid maintenance of the prescribed diets. The day before the exercise is to be done, students should begin the diet. Depending upon the quantity of urine desired, a sample taken the evening before the experimental day, or a sample collected upon awakening on the experimental day should suffice. It is not necessary to collect samples throughout the day.

TECHNIQUES

The materials listed in the *Student Laboratory Guide* are per team.

Glucose test paper is available at drugstores under many brand names. They are usually labeled as strips or papers for urine-glucose or urine-sugar tests, or some similar combination of names. Two additional tests can be added to the exercise by using combination strips (for example, Combistix, made by the Ames Co. of Elkhart, Ind.) which test for urinary protein and for pH. If test papers are used, follow closely the directions found with the papers.

Solutions of urea, glucose, sodium chloride, and pentose can be made up and mixed in varying proportions for testing in place of urine. However, in doing so the exercise loses the value of being clearly related to human physiology.

The five typical diets in the *Student Laboratory Guide* can be varied as necessary. The salt-free diet should give a low test for chloride.

The major reason for pentose testing is to show that carbohydrates in the urine may not necessarily signify diabetes mellitus. In addition, it indicates that carbohydrates may appear in the urine after the ingestion of pentose-rich food. There is a condition known as pentosuria, which is a symptomless condition that has no effects of any kind and simply involves the constant excretion of pentoses regardless of diet. Be sure that the student understands that when food is used in the body some waste products result. Protein metabolism results in nitrogenous waste products that must be removed promptly from the body to avoid poisonous effects. Some of these wastes are removed in the urine.

Just as an analysis of the exhaust of an automobile can tell us something about the efficiency and performance of the engine, so urinalysis can tell us something about human functions. If we were to burn kerosene in a car in place of gasoline, the exhaust would appear very different. So too, the diet of a human being affects the content of the urine. These points, adequately established before the exercise, will give a better motivation and insight to the student.

You may have to explain the method for the calculation necessary for the students to answer Question 1. Point out that they simply divide the total amount of the substance present in the arterial blood into the amount of the same substance remaining in the venous blood to get the percentage resorbed from the tubule.

Two test tubes can be used for each diet if they are rinsed between each test, except the urea test which requires a pair of tubes for each diet, since the test results take an hour to develop.

The use of "Invitations to Enquiry," *Biology Teachers' Handbook*, Nos. 38 through 44, dealing with homeostasis, is recommended as a method of reinforcing the theme of regulation and homeostasis.

ANSWERS TO QUESTIONS

1. Student calculations should show 99% of the water reabsorbed from the tubule, 98% of the chloride, 100% of the glucose, 33% of the urea, 87% of the uric acid, 99% of the calcium, and none of the creatinine. Therefore, all substances are not reabsorbed in equal amounts.
2. Creatinine and urea.
3. Glucose and water.
4. A large water intake would increase the volume of water in the urine.
5. A high salt content would raise the chloride content of the urine.
6. A low protein diet would decrease the amount of urea in the urine.
7. The high-protein diet should increase the urea content of the urine.
8. The salt-free diet should lower the chloride content in the urine.
9. The high-glucose diet may cause an increase in glucose in the urine.
10. The high-pentose diet will increase pentose sugars in the urine.
11. The regular diet will vary depending upon the metabolism of the individuals concerned but should not show any unusual variations.
12. They should be quite similar.
13. Because the kidney removes the excesses rapidly, the blood therefore does not reflect great changes over any considerable length of time.
14. That it has been temporarily higher than normal. The fact that glucose is appearing in the urine is an indication that sufficient quantities have been removed from the blood to maintain a normal blood glucose level. Glucose in the urine can also indicate a metabolic defect in which the body fails to break down glucose.
15. In this paragraph the student should indicate that he understands that the kidney serves a homeostatic role in removing excess substances from the blood and excreting them. He should indicate that he understands this as a differential process, that substances are selectively removed, and that without such a regulatory mechanism, the amounts of dissolved substances in the blood would vary widely.

SUGGESTED READINGS

Books

Carlson, A. J., and V. Johnson. 1961. *The Machinery of the Body.* 5th ed. University of Chicago Press, Chicago

Smith, H. W. 1953. *From Fish to Philosopher.* Little, Brown, Boston

Articles

Merrill, J. P. 1961. "The Artificial Kidney." *Sci. Am.* **205** [1] 56

Salisbury, B. F. 1954. "Artificial Internal Organs." *Sci. Am.* **191** [2] 24

FILMS

The Human Body: *Excretory System,* 1960, (sd, b&w or c, 13½ min), p, Coronet

Kidneys, Ureters and Bladder, their Structure and Function, 1947, (sd, b&w, 10 min), Bray

The Multicellular Animal, Part IV: *Excretion,* No. 6, 1962, (sd, c, 27 min), p or r, AIBS(McGraw-Hill)

The Work of the Kidneys, 1940, (sd, b&w, 11 min), p or r, EBF

Work of the Kidneys, 1950, (fs, b&w, 32 fr), p or r, EBF

ANIMAL BEHAVIOR

EXERCISE 25-1

TIME: One laboratory period.

MATERIALS: See page 191 in the *Student Laboratory Guide.*

This exercise is designed to accompany the material on nervous systems in Chapter 25 of the textbook.

■ **The purpose of this exercise is to demonstrate to the student that certain behavioral patterns are similar in widely divergent forms of animals such as man and insects.**

This exercise studies some of the behavioral characteristics of two kinds of insects, an adult fly (fruit fly) and a larval form (caterpillar) of either a moth or a butterfly. The student will come to understand that behavior involves some reactions that people do not usually think of as behavior. Note that plant movements in response to a directional stimulus are in general limited to a movement of some part of the plant and are called *tropisms.* In the case of an animal, the entire animal moves. This is called a *taxis* (plur. *taxēs*). You may prefer to avoid introducing this additional technical term, but make it clear to the students that a distinction can be made, and that technically the movements of the whole organism are called by a different name.

SPECIAL INSTRUCTIONS

In the course of this exercise, students should be encouraged to appreciate the difficulties of behavioral research and of the need for many carefully controlled experiments as a prerequisite to valid conclusions.

The problem of food location by insect larvae is, for the most part, an open-end experiment. Olfactory perception appears to be primarily responsible for the ability of larvae to locate food materials, although this has not been thoroughly established for many specific insects.

The discussion of human reflexes should be based on an understanding of simple reflex arcs and their significance in observable human behavior.

You may wish to divide students into teams to do the several parts of this exercise. Unless properly supervised, the part of the exercise concerning reflex actions in human beings tends to degenerate into a general session of horseplay by the class. You may find it advisable to demonstrate these reflex actions, especially the knee reflex and the eye reflex, to the class rather than to have the observations carried out by individual student teams. This is particularly true of the eye reflex, wherein carelessness on the part of the student performing the exercise could possibly lead to difficulty.

TECHNIQUES

The NAS-NRC sourcebook cited in Suggested Readings contains a comprehensive discussion of the fruit fly problem under the title of "Animal Behavior." It also has an additional experiment on the feeding of larvae, entitled "A Study of the Feeding Behavior of Butterfly Larvae."

If suitable larvae cannot be found in the wild state, a silkworm culture can be maintained with little difficulty. The oriental silkworm will eat mulberry leaves, osage orange leaves, and sometimes lettuce. The silkworm culture is obtainable from biological supply houses.

Tests should be conducted well beforehand to ascertain proper plant materials as food and material avoided by the caterpillar.

Blue, red, and yellow are suggested food colorings with which to dye plant materials.

It should be emphasized that because of the number of extraneous factors involved, the animal behavior may not be identical in all cases in any one class and undoubtedly will be different between different classes. Therefore, considerable latitude must be allowed in considering the student's answers to the questions in this exercise.

ANSWERS TO QUESTIONS

1. Fruit flies are positively phototactic and will move toward the light.
2. Jarring or tapping the tube will introduce a new factor which will obscure the results of phototaxis.
3. This will depend on the intensity of light, but unless the light is exceptionally strong the flies will not move toward it as readily as when the tube was 30 cm away from the light.
4. **5.** and **6.** Depend on student observation, varying with intensity of light used and numbers of flies, cleanliness of the tube, etc.
7. That the fly responds in differing degrees to different intensities of light.
8. They do not react equally strongly to lights of different colors.
9. Yes. The position of the tube will either restrict or enhance the movement of flies in certain directions.
10. Bringing the flies close to the light may bring them under the influence of heat rather than light. They will move away from excessive temperatures.
11. The experiment can involve exposure to an even illumination while placing the tube near dry ice or a hot plate. The flies will react to different temperatures, avoiding both extremes of cold and heat.
12. These are inborn reactions.
13. Individual flies can be hatched and raised separately with no contact

with any other flies. If they then react in the same fashion as the flies reared under usual conditions, one would assume the reactions are inborn, and not the result of learning from other members of the population. If the flies reared in isolation were to react in different ways from the control flies or not at all, one would conclude that the reactions are learned from other members of the fly population.

14. Dependent upon student observation, but if the caterpillar is hungry enough and external stimuli are minimized, it will move toward its food source.
15. The physiological condition of the caterpillar, including whether or not it is hungry; the conditions of the surrounding environment; the unfamiliarity of the surroundings; the distractions caused by students and other environmental stimuli.
16. The food is shifted around the circle in order to prevent any accidental location of the food because of the caterpillar's preference for taking a particular direction. In other words, the caterpillar may be responding to light, heat, or other factors that make it move preferentially in one direction. This might mistakenly be interpreted as movement toward a certain type of food lying in that direction.
17. Variable; to be determined by the student's observation.
18. If it were removed a considerable distance from the food source it would be able to find food only by accident.
19. To be determined by the student. Color will play little part in the selection.
20. The caterpillar finds its food mainly by olfactory sense, and therefore obscuring the odor tends to prevent the larva from locating its appropriate food source.
21. The shape of food material has little effect on the way in which the caterpillar finds its food.
22. Primarily by odor.
23. No. Many other factors may enter in. Negative geotaxis, for example, leads the animal to climb plants, and other factors may influence the way this particular type of caterpillar locates its food.
24. The foreleg (shank of the leg) is extended.
25. Yes. The harder the knee is struck, the greater the degree of response.
26. No. The reflex reaction is highly variable.
27. It is a natural innate reaction.
28. No conscious effort is required for performing a reflex action.
29. Yes. The reflex can be repressed by deliberate contraction of the muscles in the upper leg that normally flex the foreleg.
30. The subject blinks.
31. This is an innate behavioral characteristic.
32. Yes. The individual can become adapted to the stimulus, and then does not blink.
33. The pupil that has been covered enlarges in the dark, to increase the amount of light entering the eye. The pupil left uncovered does not change.

34. There should be no indication of feeling in this reaction.
35. Both eyes should respond the same way.
36. The reflex responses of human beings and the responses observed in flies and caterpillars are similar in being innate and apparently in requiring no conscious effort.

SUGGESTED READINGS

Books

Carr, A. 1961. *Guideposts of Animal Navigation*. BSCS Pamphlet No. 1. D. C. Heath

Morholt, E. 1958. *A Sourcebook for the Biological Sciences*. Harcourt, Brace & World, New York

National Academy of Sciences-National Research Council. 1957. *Laboratory and Field Studies in Biology: A Sourcebook for Secondary Schools*. Washington, D. C.

Articles

Best, J. B. 1963. "Protopsychology." *Sci. Am.* **208** [2] 54

Bazier, M. A. B. 1962. "The Analysis of Brain Waves." *Sci. Am.* **206** [6] 142

Blough, D. S. 1961. "Experiments in Animal Psychophysics." *Sci. Am.* **205** [1] 113

Dilger, W. C. 1962. "The Behavior of Lovebirds." *Sci. Am.* **206** [1] 88

Eibl-Eibbsfeldt, I. 1961. "The Fighting Behavior of Animals." *Sci. Am.* **205** [6] 112

Frisch, K., von 1962. "Dialects in the Language of Bees." *Sci. Am.* **207** [2] 78

Harlow, H. F. and M. D. 1962. "Social Deprivation in Monkeys." *Sci. Am.* **207** [5] 136

Hess, E. H. 1961. "Shadows and Depth Perception." *Sci. Am.* **204** [3] 138

Hodgson, E. S. 1961. "Taste Receptors." *Sci. Am.* **204** [5] 135

Holst, E., von, and U. von St. Paul. 1962. "Electrically Controlled Behavior." *Sci. Am.* **206** [3] 50

Kortlandt, A. 1962. "Chimpanzees in the Wild." *Sci. Am.* **206** [5] 128

Melzack, R. 1961. "The Perception of Pain." *Sci. Am.* **204** [2] 41

Miller, W. H. *et al.* 1961. "How Cells Receive Stimuli." *Sci. Am.* **205** [3] 222

Washburn, S. L. and I. DeVore. 1961. "The Social Life of Baboons." *Sci. Am.* **204** [6] 62

FILMS

The Multicellular Animal, Part IV: *Behavior*, No. 11, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

The Multicellular Animal, Part IV: *The Animal and the Environment*, No. 12, (sd, c, 25 min), p or r, AIBS(McGraw-Hill)

Baboon Behavior, 1961, (sd, c, 31 min), p or r, UCLA

Behavior in Animals and Plants, 1957, (sd, b&w or c, 11 min), p, Coronet

The Ears and Hearing, 1950, (sd, b&w, 10 min), p or r, EBF

Endocrine Glands, 1939, (sd, b&w, 11 min), p or r, EBF

How the Ear Functions, 1941, (sd, b&w, 11 min), KB

Human Brain, 1954, (sd, b&w, 11 min), p or r, EBF

Principles of Endocrine Activity, 1960, (sd, b&w or c, 16 min), p or r, Indiana

CONTROL OF MUSCLE CONTRACTION

EXERCISE 26-1

TIME: One laboratory period.

MATERIALS: See page 195 in the *Student Laboratory Guide.*

This exercise is designed to accompany the material on cardiac and smooth muscle in Chapter 26 of the textbook.

■ **The purpose of this exercise is to determine the effects of the secretions of the nervous system on the regulation of muscular contraction in cardiac and smooth muscle.**

SPECIAL INSTRUCTIONS

This exercise has been designed to show that chemical processes are involved in nerve impulses and to show the responses of cardiac and smooth muscle to these chemical stimuli. These muscle activities are regulated by substances released by the autonomic nerve endings which innervate them (sympathetic nerves release an adrenalinelike substance; parasympathetic nerves release acetylcholine). Each of these muscle tissues is regulated by both divisions of the autonomic nervous system. The effects of the two divisions are antagonistic. In this particular exercise, sympathetic and parasympathetic stimulation (simulated by using adrenaline and acetylcholine, respectively) produce opposite effects on the activity of the heart and of smooth muscle. Adrenaline accelerates the rate of heartbeat and inhibits the stomach. Acetylcholine inhibits the rate of heartbeat and accelerates stomach activity. Notice that adrenaline is accordingly not a uniform stimulant throughout the body, and neither is acetylcholine uniformly an inhibitor.

If too much acetylcholine is added the smooth muscle will go into a spasm, and thus give students the erroneous impression that it was inhibited, when it was really exhibiting a maximal sustained response. The heart may also stop, in which case the response in both would apparently be the same in both types of muscle, although actually for opposite reasons. If these effects should occur, the situation can be used to advantage by raising the questions: (a) Was the response really the same in both tissues? (b) How could this be determined?

Four "Invitations to Enquiry," Nos. 32, 33, 34, and 35 in the *Biology Teachers' Handbook* use muscle as an exemplar of the concept of function and are recommended for use in conjunction with this exercise. Invitation 15 uses the neurohormones of the heart to deal with origin of scientific problems.

TECHNIQUES

This exercise can be used as a demonstration, or the class can be divided into teams of various sizes. The exercise is also suitable for use as an individual project.

The adrenaline and the acetylcholine can be obtained from a local physician or in vials of 20 ml or less of 1:1000 concentration from drug supply houses.

It would be a good idea to have several extra frogs on hand to replace tissues that may fail to respond because of muscle fatigue.

The frogs for this exercise should be pithed according to the technique described in Exercise 20-3.

ANSWERS TO QUESTIONS

1. Depends on student observation.
2. Depends on student observations, but contractions may be noticed.
3. The heart will be more active in Ringer's solution than the stomach.
4. Depends on student observation, but it should increase over the rate reported in Question 1, above.
5. Depends on student observation, but it should be slower than any reported in Question 2, above.
6. Depends on student observation, but it should be less than observed in Question 1, above.
7. The contractions of the stomach should be more frequent and more noticeable in the acetylcholine solution than in either Ringer's or adrenaline solutions.
8. The temperature increase should increase the rate of heartbeat.
9. The temperature increase should increase the rate of stomach contraction.
10. The lowered temperature should decrease the rate of heartbeat.
11. The rate and force of stomach contractions should decrease.
12. The beat should be disturbed and reduced by the differences in the salt content between the tissue and the external solution. Ringer's solution contains salts that are not present in a simple NaCl solution.
13. There should be a reduction and disturbance of the contractions, ultimately ending in death of the tissue.
14. No. They respond in opposite ways.
15. No. They respond in opposite ways.
16. It would increase the rate of heartbeat.
17. It would decrease the rate and force of stomach contractions.
18. It would decrease the rate of heartbeat and increase the rate of stomach contractions.
19. Yes, a higher temperature increases the rate of reaction. This will continue until a temperature slightly in excess of 37° C is reached, at which time the tissue will fail to respond further.

20. Yes. Both tissues slow down their reactions as the temperature is lowered. This will continue until the freezing point is reached.
21. Because they produce opposite results. One is needed for acceleration. The stimulation of the other is needed for inhibition. Were only one type of nerve to be present, the heart could only be accelerated and not slowed; or inhibited but not accelerated.
22. Adrenaline, because one would expect it to increase the rate of heartbeat—to stimulate the heart's action.

SUGGESTED READINGS

Books

Hoyle, G. 1957. *Comparative Physiology of the Nervous Control of Muscular Contraction.* Cambridge Monographs in Experimental Biology, No. 8. Cambridge Univ. Press, London

Articles

Anon. 1951. "Heart Muscle." *Sci. Am.* **185** [2] 48
Astbury, W. T. 1951. "Flagella." *Sci. Am.* **184** [1] 20
Dean, G. 1957. "Pursuit of a Disease." *Sci. Am.* **196** [3] 133
Gerard, R. J. 1948. "The Dynamics of Inhibition." *Sci. Am.* **179** [3] 44
Harary, I. 1962. "Heart Cells *in vitro.*" *Sci. Am.* **206** [5] 141
Hayashi, T., and A. W. Boehm. 1952. "Artificial Muscle." *Sci. Am.* **187** [6] 18
Katchalsky, A., and S. Lifson. 1954. "Muscle as a Machine." *Sci. Am.* **190** [3] 72
Szent-Gyorgyi, A. 1949. "Muscle Research." *Sci. Am.* **180** [6] 22

FILMS

The Multicellular Animal, Part IV: *Muscles,* No. 8, 1962, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)
The Muscular System, 1951, (sd, b&w, 11 min), p or r, UW
Moving X Rays, 1945, (sd, b&w, 10 min), p or r, Almanac
Frog Heartbeat, 1960, (sd, c, 7 min), p or r, Thorne
Frog Skeletal Muscle Response, 1961, (sd, c, 4 min), p or r, Thorne
Turtle Heart Neural Control, Thorne (Soon to be released.)
Work vs. *Load in Frog Skeletal Muscle,* Thorne (Soon to be released.)

EFFECTS OF REPRODUCTIVE HORMONES

EXERCISE 27-1

TIME: One period to perform the exercise, plus parts of four subsequent days for observation.

MATERIALS: See page 197 in the *Student Laboratory Guide.*

This exercise should accompany the section on the reproductive hormones in Chapter 27 of the textbook.

■ **The purpose of this exercise is to have the students determine the effect of testosterone, applied in different concentrations to male chicks, on the development of combs and male behavior patterns, including crowing—also, to compare the effects of this hormone when applied externally, to the effects when it is injected subcutaneously.**

SPECIAL INSTRUCTIONS

Students can be divided into teams for the performance of this exercise. Number of students per team will depend on class size and availability of materials.

Chicks of any age group may be used for this exercise, but the use of day-old cockerels is recommended. Day-old birds can be procured from local hatcheries at a cost of only a few cents a bird, while older birds are more expensive. *Be sure to get sexed chicks known to be males.* When starting with day-old chicks the results are more pronounced and dramatic. Some of these will be crowing and fighting within 5 days after injections are started. Combs will show a rapid development within this period. There will be a very noticeable contrast between the treated and the control groups of birds.

You can arrange housing so that the chicks take up very little space. They can be housed in empty aquaria or open-topped cardboard boxes. A light bulb suspended within each housing unit will provide adequate heat. The boxes need not be more than 20 cm high. Cover the bottom of the box with a light layer of pine shavings and provide containers for food and water. Feed the usual chick mash, available at any feed store or hatchery. Discard and replace soiled shavings periodically.

This exercise has been designed to run for 5 days, and so should be begun on a Monday. It can very easily be extended for additional days. The crowing behavior will be more pronounced and probably will occur in more chicks if the exercise is prolonged a few more days.

TECHNIQUES

Testosterone can be obtained from a biological supply house. Testosterone may be supplied in an oil vehicle and can be diluted with nonsterile corn oil obtainable from the grocery. Testosterone propionate or a similar compound, already in water solution, may be substituted.

Make a stock solution of 50 mg per ml. An injection of 0.1 ml of this solution will contain 5 mg and this, given for five successive days, will total the 25 mg needed for the 25-mg test. The 500-mg solution can be diluted to make the 150 mg per ml and 50 mg per ml solutions which will give the 15 and 5 mg totals needed for the other tests. Note the following summary of dilutions and injections:

Solution	***Per Injection***	***Five-day Total per Chick***
10 mg per ml	1 mg in 0.1 ml	5 mg
30 mg per ml	3 mg in 0.1 ml	15 mg
50 mg per ml	5 mg in 0.1 ml	25 mg

The comb index is described as being height times length times width. This is preferred because it involves more measuring. A simpler formula, which you may use if you desire, is height times length divided by 2. This latter formula decreases the chance of error, and it is used by a number of endocrinologists. The students may question how to measure the comb, but as they are the scientists in this experiment they should determine this for themselves. It should be relatively easy to determine the length of the comb at its base, its height at its highest point, and the greatest width at the base.

Ask the students to speculate on the effects of using estrogen rather than testosterone in this exercise.

At the end of this exercise many teachers have given experimental animals to students to take home. We do not feel this is desirable, particularly in view of the use of endocrine hormones that may affect the edibility of the bird. The surplus chicks remaining after this exercise should be killed by the teacher by administering a lethal dose of anesthetic or by other humane means. This procedure should be performed out of view of the class. After killing, body weight and testes weight may be obtained and added to the other data of this exercise.

The food-coloring dyes are easily obtained and serve to mark the chicks without harming them.

A sample exercise data sheet is included in case you may wish to reproduce it in mimeograph form or on the board.

ANSWERS TO QUESTIONS

1. The control group provides data for comparison with the experimental group. Unless there is a control group differing from the experimental group by only a single condition, one cannot logically conclude that any difference observed between the groups results from any particular cause or condition.
2. Depends on student data; but the chicks receiving the subcutaneous injections should show the greatest difference.
3. Depends on student data; but generally the greater the testosterone concentration, the greater the comb development.
4. The greater the concentration of the testosterone, the greater the comb development; but there is less development than when the hormone is injected subcutaneously.
5. The chicks that received the highest concentrations subcutaneously.
6. Since caponized roosters have their testes removed, injecting testosterone into these capons would bring about a development of male characteristics such as renewed comb growth and crowing.

SAMPLE EXERCISE DATA SHEET

			1st Day		2nd Day, etc.
		Average Comb Index	Behavior Observations	Average Comb Index	Behavior Observations
Team A 5 mg	Control				
	Injection				
	Comb application				
Team B 15 mg	Control				
	Injection				
	Comb application				
Team C 25 mg	Control				
	Injection				
	Comb application				

SUGGESTED READINGS

Books

Rugh, R. 1948. *Experimental Embryology: A Manual of Techniques and Procedures*. Burgess, Minneapolis

Turner, C. D. 1955. *General Endocrinology*. W. B. Saunders, Philadelphia

Articles

Choh, H. L. 1950. "The Pituitary." *Sci. Am.* **183** [4] 18

Gray, G. W. 1957. "The Organizer." *Sci. Am.* **197** [5] 79

Zuckerman, S. 1957. "Hormones." *Sci. Am.* **196** [3] 77

FILMS

The Multicellular Animal, Part IV: *Hormones*, No. 7, 1962, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

Contains a segment using a rooster and a capon.

The Multicellular Animal, Part IV: *Behavior*, No. 11, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Shows the hormones of the endocrine glands affecting behavior in birds, mammals, fishes, etc.

REPRODUCTION AND DEVELOPMENT IN THE FROG

EXERCISE

28-1

TIME: Preparatory time of up to 48 hours, plus one or two laboratory periods for observation plus parts of 11 subsequent days for periodic observations.

MATERIALS: See page 199 in the *Student Laboratory Guide*.

This exercise is to accompany the section on amphibian development in Chapter 28 of the textbook.

■ **The purpose of this exercise is to present the effects of reproductive hormones on ovulation and sperm release in the frog. The student will also have an opportunity to examine the gametes of the frog, to perform an artificial fertilization of frog eggs, and to observe the subsequent development of a frog.**

SPECIAL INSTRUCTIONS

The time required for this exercise can be considerably shortened if some preparations are made previous to class. For example, in planning Part A 48 hours must elapse after injection before ovulation will take place. Therefore, it is possible to inject on a Friday and have eggs available on a Monday. Part B requires a wait of 2 hours after injection to take the sperm smears. This means that the same class cannot inject and also take smears from the same frog and that some frogs will have to be injected before the first classes in the morning for sperms to be available. Later classes can use those frogs injected in the earlier morning classes. Parts C and D can be taken together. Part D requires up to 48 hours of prior preparation, as in Part A, with an important observation 2½ hours after injection. If the students are to watch development of the embryos, periodic observations for 11 subsequent days will be required for Part D. It will be necessary for the teacher to schedule this exercise carefully to make the most efficient use of materials throughout the day.

The simplest method of inducing ovulation in the frog is to purchase, from a biological supply house, a kit which contains the necessary male frogs, female frogs, and a bottle containing a suspension of pituitaries. Merely inject the suspension, using a No. 25 hypodermic needle, into one of the female frogs and it will usually ovulate.

However, to present this exercise as a mere demonstration would mean the loss of a very important aspect of the experiment. Students should dissect out the frog pituitaries. This is a difficult procedure for students to follow, so the BSCS staff has prepared a short 16-mm sound film showing how to remove a frog's pituitary. The use of this film depends upon the objectives of each individual teacher. However, it is a good supplemental teaching aid to accompany this exercise. Most of this laboratory work can be done by student pairs or teams rather than as a demonstration. If you have students dissect out the pituitary, the following will be required:

One female frog for each team plus three extra frogs for each class
Chloroform; or 2% urethane or 0.1% chloretone solution
Fine-pointed scissors
Dissecting needle
Dissecting forceps with fine points
Dissecting pan
Watch glass
Saline solution (3.5 g of NaCl per liter of water)
Hypodermic syringe (5 ml or smaller)
Hypodermic needle, No. 18 or No. 20

Using the above materials, the procedure is described below under the heading, Techniques.

"Invitations to Enquiry," No. 25, entitled "Pituitary-Gonad Mechanism," in the *Biology Teachers' Handbook* will be of value as an additional source of material for the teaching of this laboratory exercise.

It is essential for these experiments that glassware be clean and free from residual chemicals. If possible, new glassware, which has had nothing more than distilled water in it, should be used for this exercise. The presence of even small amounts of foreign chemicals can interfere with the success of this exercise.

If the doses recommended do not work, try increasing the dosage.

Seasonal effects are important. The frog test does not work accurately in the late summer months.

TECHNIQUES

Using the materials mentioned under Special Instructions, above, the procedures for dissecting out the frog pituitary are as follows:

Render a live frog unconscious by anesthetizing it with ether, chloroform, a solution of 2% urethane in water, or a solution of 0.1% chloretone in water. To kill the frog, decapitate it by inserting a blade of a strong, sharp-pointed scissors into the mouth at the angle of the jaw, cutting back to extend the width of the mouth, and making a transverse cut just posterior to the tympanic membranes.

You have now cut off the upper jaw and skull as far back as the rear of the tympanum. Keep this piece and discard the body, after pithing the carcass by inserting a dissecting needle in the spinal column and moving it about to destroy the spinal cord and thus prevent further movement of the frog. Wash the head and place it, ventral (oral) side up, on the dissecting pan. Cut away the skin of the roof of the mouth to expose the cross-shaped bones of the skull.

Carefully insert the scissors in the opening at the base of the brain and make two cuts in the bony floor of the cranium. Cut down one side to a point even with the anterior margin of the eye socket and do the same on the other side of the floor, making sure the cuts are approximately 2.5 mm to either side of the midline. Using forceps, carefully turn back the flap of bone to expose the underside of the brain. The pinkish pituitary gland is attached to the brain just posterior to the crossed optic nerves (optic chiasma) and is about three times the size of a straight-pin head. Usually the pituitary adheres to the bony flap which has been turned back. Remove it with fine forceps, to prevent mashing it, and place it in a small amount of the saline solution in a watch glass.

The number of pituitaries required to induce ovulation in a single female varies with the sex of the donor and the season. Pituitaries from female frogs are approximately twice as potent as those from males. The dosage of female pituitaries required to induce ovulation in a female frog from September to January is five glands; February, four glands; March, three glands; and April, two glands. However, to assure success it is suggested that five pituitaries from females be used at all times.

Although the purchase of female frogs is suggested above, some of you may have the opportunity to collect your own frogs and may wish to separate the two sexes. Several external characters distinguish the female from the male frog (see Figure 3-6-1, page 18). The skin of the enlarged abdomen of the female is creamy white and usually lacks any mottling of black pigment. The male of comparable length has a more slender abdomen and the ventral skin is mottled. During the mating season the male has an enlargement at the base of the thumb which is absent in the female. This feature is recognizable, although not so easily, at other times of year. The tympanic membrane of the female is somewhat smaller than that of a comparable male.

Place the pituitaries from five females in a watch glass. Remove the needle from the syringe and draw all of the whole glands, along with 1 ml of solution, into the barrel of the syringe. Expel any air in the syringe, attach a No. 16 or No. 18 needle, and you are ready to inject the glands into the abdominal cavity of the female frog. It is important to avoid the vital organs of the frog, so with the thumb and forefinger pinch up the skin and belly muscle and insert the needle along this fold, being certain that the tip of the needle advances only to the proper depth. It is a good idea to make a second injection of a few milliliters of solution to make certain that no pituitary glands remain in the shaft of the needle.

For Part B, Antuitrin "S" (chorionic gonadotrophin) can be purchased from Parke, Davis Co., Detroit, Mich. It is supplied in dry form along with a 10-ml ampule of physiological saline solution. One package will supply 5000 international units per 10 ml when mixed.

The frog supply is probably the most acute problem in this exercise. Some schools can secure live frogs from local suppliers at very reasonable cost (e.g., from Steinhilber & Co., or Hoffman Bros., both companies in Oshkosh, Wis.). Frogs may be obtained from the large biological supply houses, but at greater expense.

To minimize the difficulty and expense of obtaining frogs, there are some possible solutions. If live frogs are used earlier in the year for other laboratory experiments, the pituitaries can be collected from these specimens and stored in water or 10% ethyl alcohol in the freezing compartment of a refrigerator for as long as 2 months.

Live frogs may be collected during the early fall and kept for several weeks simply by storing them in wet sphagnum moss or paper towels lining the meat compartment of a refrigerator kept at 9° C (48° F). If the moss gets soiled, wash it in hot water, wring it out, let it cool, and then put it back with the frogs. The paper towels may simply be discarded and replaced with new ones.

Sometimes frogs can be maintained for periods longer than several weeks by keeping them in a clean washtub (use no detergent) filled with a little water to which some sulfanilamide is added. A few rocks are added as islands upon which the frogs may gather. The tub should be scrubbed (use no detergent) and flushed out twice a week. Add about an inch of water to the fresh tub and sprinkle sulfanilamide into the water until there is a thin film covering it. The main hazard in trying to keep frogs is that they usually become infected with a fungus disease called "red-leg" which causes the breakdown of blood vessels in the legs and quickly causes death. The sulfanilamide should prevent this disease. The tub should be covered with a screen of 13-mm or ½-inch hardware cloth. The frogs should not be fed.

For Parts B, C, and D the following techniques will prove valuable.

If you wish to use in Part D the sperm suspension obtained in Part C of this exercise, it will be necessary to combine Parts C and D during the same laboratory period. Part A must of course be done one or two days previously. Part C should be allowed approximately 20 minutes and Part D the remaining time. Time is such a critical factor in this exercise that the students should read all parts of the directions before coming to the laboratory. All laboratory materials should be made available at some central location or distributed previously to each laboratory station. Part B is optional and is not a satisfactory source of sperms for Part D.

To save further time, take the pituitary-treated females and milk out a few eggs. Discard these first eggs, because they will probably have absorbed too much body fluid to be fertilized. The remaining eggs may be stripped into a shallow pan while the students are preparing their sperm suspensions.

If you decide to leave the fertilized eggs on the tables for observation of further development, there are certain precautions to be taken. The pond water must be changed as often as possible, at least every other day. Use pond water or aerated tap water. Keep the temperature below 25° C. Do not keep more than 30 to 40 eggs in a dish. All eggs that are not developing or were not fertilized should be removed immediately. The success of continued growth will be limited unless these precautions are observed. The surface area of the individual dishes is small and consequently lack of aeration and the build-up of toxic materials from the metabolism of the developing embryo take their toll. Large containers with more water and a greater surface area will give better results than small dishes. Plastic 1-gallon ice cream containers are excellent and inexpensive.

Several variables that will make interesting controlled experiments can be introduced during the development of the frog eggs. The factors which can be controlled most easily are the amount of oxygen available and the temperature for proper development. These experiments are explained in the BSCS laboratory block entitled "Animal Growth and Development," written by Dr. Florence Moog.

ANSWERS TO QUESTIONS

1. Students will detect sperm movement and perhaps can see that the beating of the tail is the cause of this motion. They may compare sperm locomotion to the movement of a tadpole.
2. No. The methylene blue stain will kill the sperms.
3. The tail should now be visible.
4. The stain kills the sperm but makes the tail visible.
5. Externally, more than half the egg appears densely black due to surface pigment granules, while the rest is creamy white. A jellylike mass surrounds each almost round egg and gives it the appearance of a small bead enclosed in a transparent covering.
6. The orientations of the dark and light sides of the eggs indicate random positions of the eggs.
7. The jelly helps protect the egg against mechanical injury, against

ingestion by some organisms, and against fungus and other infections. Equally important is that this jelly helps the egg retain its own heat, so that the jelly may be said to act as an insulator against heat loss. But the jelly does not concentrate the heat rays of the sun onto the egg, as is sometimes claimed. It is largely water and a poor conductor of heat.

8. Each egg becomes separated from the others by a thick protective layer as the jelly swells, and the eggs rotate so that the heavy, yolk-laden area is directed downward. Rotation occurs when the newly fertilized egg splits away from the membranes which previously held it tight. About an hour after fertilization, the gray crescent also appears.
9. The first cleavage furrow appears, which indicates that the egg is beginning its development.

SUGGESTED READINGS

Books

Rugh, R. 1948. *Experimental Embryology: A Manual of Techniques and Procedures.* Burgess, Minneapolis

Articles

Dunn, L. C. 1950. "Genetic Monsters." *Sci. Am.* **182** [6] 16
Gamow, G. 1955. "Information Transfer in the Living Cell." *Sci. Am.* **193** [4] 70
Goldschmidt, R. B. 1949. "Phenocopies." *Sci. Am.* **181** [4] 46
Ingalls, T. H. 1952. "Mongolism." *Sci. Am.* **186** [2] 60
Kalmus, H. 1952. "Inherited Sense Defects." *Sci. Am.* **186** [5] 64
Lüscher, M. 1953. "The Termite and the Cell." *Sci. Am.* **188** [5] 74
Pincus, G. 1951. "Fertilization in Mammals." *Sci. Am.* **184** [3] 44
Reynolds, S. R. 1952. "The Umbilical Cord." *Sci. Am.* **187** [1] 70
Shumway, W. 1940. "Stages in the Normal Development of *Rana pipiens.* I. External Form." *Anatomical Record.* **78** 139
_____ 1942. "Stages in the Normal Development of *Rana pipiens.* II. Identification of the Stages from Sectional Material." *Anatomical Record.* **83** 309
Stern, C. 1952. "Man's Genetic Future." *Sci. Am.* **186** [2] 68
Stone, A. 1954. "The Control of Fertility." *Sci. Am.* **190** [4] 31
Waddington, C. H. 1953. "How Do Cells Differentiate?" *Sci. Am.* **189** [3] 108
Weisz, P. B. 1953. "The Embryologist and the Protozoan." *Sci. Am.* **188** [3] 76

FILMS

Life Cycle of the Frog, 1950, (sd, b&w, 11 min), p or r, UW
The Multicellular Animal, Part IV: *Behavior,* No. 11, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)
The Reproductive Processes of the Frog, Rana pipiens, 1948, (si, b&w, 45 min), Roberts Rugh
Removing Frog Pituitary, 1960, (sd, c, 1 min), p or r, Thorne
Reproduction, Growth and Development, Part V: *Theories of Development,* No. 5, 1962, (sd, c, 30 min), p or r, AIBS(McGraw-Hill)

CHICK DEVELOPMENT

EXERCISE
28-2

TIME: A minimum of two laboratory periods with lead time for the incubation of eggs up to 21 days.

MATERIALS: See page 203 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on the development of amniotes in Chapter 28 of the textbook.

■ **The purpose of this exercise is to observe the stages in the development of the chick, an animal with embryonic membranes, and to compare and contrast this development with that of the frog seen in Exercise 28-1.**

SPECIAL INSTRUCTIONS

This exercise has been adapted from the BSCS block "Animal Growth and Development," by Dr. Florence Moog. You may refer to the most recent edition of this block for experiments with developing chicks.

The time required to do all five parts of this exercise can be confined to two periods only if you have planned to provide several stages for use on the same day.

Most local hatcheries will provide eggs pre-incubated to any desired stage, and thus it may be possible to obtain on order the various stages that are required on any particular day. However, students enjoy the experience of incubating the eggs in the laboratory and should be given the opportunity to do so if at all possible.

The use of "Invitations to Enquiry," No. 37, in the *Biology Teachers' Handbook* is recommended. This invitation deals with embryonic circulation as a means of demonstrating persistence as evidence of function.

TECHNIQUES

Teachers are frequently asked the function of the albumen of the egg. Students should be informed that it is a source of both water and protein for the embryo.

It is not necessary to fix (preserve) embryos as suggested at the end of each part of this exercise, but preserving the embryos makes them available for later independent projects. Students might make whole mounts of chick embryos, for example.

Incubators must be carefully checked in advance for constancy of temperature and control of humidity. The latter is the most common source of trouble in incubating eggs. It is recommended that the electrical plugs on incubators be taped into the socket to avoid costly accidents resulting from accidental unplugging of the incubator.

It is advisable to candle eggs incubated by the class at about 48 hours so that no infertile eggs will be given to students. A simple device for candling can be made by placing an electric lamp in a tin can which has had a hole, slightly smaller than the diameter of an egg, cut into it. By holding the egg up to this hole, development of the embryo or lack of it can be seen easily.

ANSWERS TO QUESTIONS

The answers to these questions may vary slightly depending on whether the egg has been incubated for 10, 14, 18, or 21 days. However, the general pattern given in the following answers is a point of departure.

1. Yes, the heart begins to beat at about 44 hours of incubation.
2. Yes, signs of blood are seen as early as the 26th hour of incubation. Blood islands are present in the posterior half of the blastoderm.
3. The blood vessels, which ramify on the yolk, converge toward the embryo from all parts of the vascular area, and empty into veins which return the blood to the region near the heart. The vessels which leave the embryo and ramify on the yolk do so near the posterior end of the dorsal aorta.
4. The red blood cells are formed within the first 2 days of incubation. The white blood cells appear later in development. The first granulocytes can be recognized in the bloodstream on the 3rd day. Polymorphonuclear leucocytes first appear on the 5th day. Eosinophils have been seen in exceptional cases as early as the 7th day. Basophils have been noted as early as the 14th day. Lymphocytes are first found in the blood of the 17-day embryo. The student will probably see individual red blood cells and may see a white cell or two.
5. The student will undoubtedly comment on the increase in the size of the head region, the more prominent eye, the general flexion of the anterior part of the body to the right, a general growth of the body, the enlarged and more prominent heart, the expansion from the 16-somite stage at 48 hours to 30-36 somites, extending beyond the level of the leg bud.
6. The amnion is filled with a watery fluid.
7. In the 5-day embryo there is a definite neck separating head from trunk.
8. The embryo has a tail which extends beyond the posterior limb bud.
9. Both limb buds at this stage look essentially similar and will more likely suggest legs than wings.
10. The embryo is still sealed in its fluid-filled amnion.
11. A vascular sac still surrounds the yolk.
12. The yolk stalk connects the yolk to the embryo.
13. The large arteries and veins.

14. The head, beak, feet, and wings make the embryo look like a bird. In addition, at later stages, feathers can be seen.
15. The head is abnormally large. The wings are very tiny.
16. At about 10 days it would be difficult to recognize it as a chicken. By 21 days it looks more chickenlike, but the student would nevertheless probably be correct in answering that it looked like birds in general.
17. At about 10 days the eyelids have just begun to develop. By 21 days they are completely developed.
18. Indications of feathers can be seen at the 10th day and are obvious as early as 13 days.
19. The legs are disproportionately large in all stages from the 10th day through the 21st day.
20. There are scales on the feet, becoming more pronounced when the chick is older.
21. There are claws on the toes. As early as the 10th day, claws are just visible on the termini of the toes and on digit 1 of the wing.
22. No. It does not poke its beak into the air space.
23. Yes. It gets its air supply through the allantois.
24. The chorioallantois is sticky.
25. The egg tooth and the hatching muscle on the back of the neck aid the chick in escaping from the egg.
26. By 19 to 20 days the yolk sac is half enclosed in the body cavity and may still be apparent even at the time of hatching.

SUGGESTED READINGS

Books

Hamilton, H. 1952. *Lillie's Development of the Chick.* Holt, Rinehart and Winston, New York

Hamburger, V. 1960. *A Manual of Experimental Embryology.* (Rev. ed.) University of Chicago Press, Chicago

Moog, F. 1963. *Animal Growth and Development.* BSCS laboratory block (Rev. ed.) D. C. Heath, Boston, Mass.

Patten, B. 1951. *The Early Embryology of the Chick.* 4th ed. McGraw-Hill, New York

Sussman, M. 1960. *Animal Growth and Development.* Prentice-Hall, Englewood Cliffs, N.J.

FILMS

Development of a Bird Embryo, 1932, (si, b&w, 11 min), p or r, EBF

Development of the Chick, 1950, (sd, b&w, 11 min), p or r, UW

Development of the Chick Embryo, 1953, (sd, b&w or c, 5½ min), p, Coronet

Principles of Genetics, No. 32: *Developmental Genetics* I, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Life Before Birth, 1960, (sd, b&w, 26 min), p, Carousel

Chick Embryo Explantation, 1959, (sd, b&w, 8 min), p or r, Thorne

Reproduction, Growth and Development, Part V: *Development of Organs*, No. 7, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

REGENERATION

EXERCISE

29-1

TIME: One laboratory period plus subsequent observation periods over 2 weeks.

MATERIALS: See page 209 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on regeneration in Chapter 29 of the textbook.

■ **The purpose of this exercise is to experiment with regeneration in an invertebrate animal (planarian) and a vertebrate animal (tadpole) and to compare and contrast the process in both.**

SPECIAL INSTRUCTIONS

Planarians may be purchased from supply houses or collected in nature. Some species do not regenerate well so the exercise should be pretested if field-collected animals are used.

We have attempted to introduce an investigatory flavor into the exercise by inducing the student to design experiments of Types 4, 5, and 6. Figure 29-1-1 on the following page shows some ways of obtaining these results. The teacher or the brighter students will undoubtedly think of other methods that can be explored.

Tadpoles of any age may be used. However, if they are too young, they are too small for good work; and if they are too old, care must be taken to avoid cutting off the whole tail since animals will bleed to death from the large blood vessels. In older tadpoles the cut must be restricted to the tail fin only, either on the edge or the end of the tail.

The teacher may need to experiment with the necessary concentration of anesthetic. An average useful concentration is 1 : 2500 but needs vary with age of tadpoles and local conditions. Regeneration will take from 10 days to 2 weeks.

TECHNIQUES

Animals should be kept in a cool dark place but not refrigerated, and fed about twice a week on small pieces of uncooked liver, either fresh or frozen. After about two hours of feeding, the liver should be removed and the water changed. Do not feed the animals for at least a week before using them for the regeneration experiments. Undigested food is released from the gut during cutting and will decay and kill the regenerating animals.

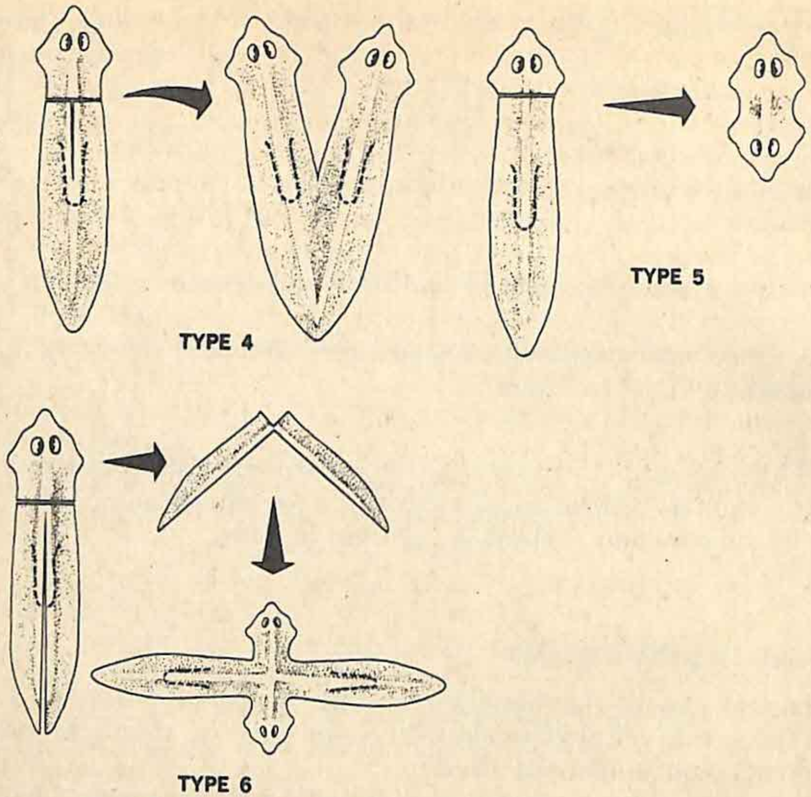


Figure 29-1-1 **Additional operations for regeneration of planarians.**

The chief causes of the high mortality in planarian work are undoubtedly keeping animals in a room which is too warm, and toxic decay products from food or dead animals. Recall that these animals are adapted to flowing, fresh-water streams where neither of the above conditions prevails.

Tadpoles from earlier exercises will probably not be old enough for this experiment. They must either be collected or ordered from a supply house and they must be of an older-aged stock than those used for Exercise 28-1.

Feed the tadpoles baby-food spinach. Other foods are satisfactory but do not work quite as well.

ANSWERS TO QUESTIONS

1. Normally within 2 or 3 days.
2. The new cells are unpigmented.
3. Anterior ends regenerate heads, posterior ends, tails. Regeneration is faster at the anterior end.
4. The initial regeneration bud is crooked on an oblique cut but differential growth soon straightens it out so that the resulting animal is not crooked. This poses an interesting research problem which is not yet solved.

5. In general, there is a gradient in the speed of regeneration and also a gradient of perfection of regenerated structure—both greater the nearer the cut is made to the head.
6. Heads may be small and irregular in shape, lacking auricles, or having no eyes, one eye, small eyes, etc. Tails may be crooked or incomplete. Pigmented patterns may be abnormal.
7. In large pieces the polarity is maintained and heads and tails form at appropriate ends. In short pieces, as in operations of Types 5 and 6, there is apparently not enough difference between the levels of cut surfaces for polarity to be maintained and bizarre results are frequent, most often heads at both ends. An anterior piece is unlikely ever to form a tail.
8. The wound heals very rapidly, within a few hours.
9. Within a few days the first signs of regeneration appear.
10. The pigment appears in about a week or so.
11. Yes, at first. But then the piece straightens as it does in the regenerating planarian.
12. They are often frilly. They may be crooked and pigment abnormalities are frequent.

SUGGESTED READINGS

Books

Child, C. M. 1941. *Patterns and Problems of Development.* University of Chicago Press, Chicago

Hamburger, V. 1942. *A Manual of Experimental Embryology.* University of Chicago Press, Chicago

Rudnick, D. 1962. *Regeneration.* Ronald Press, New York

Articles

Best, J. B. 1963. "Protopsychology." *Sci. Am.* **208** [2] 54

Rose, S. M. 1949. "Transformed Cells." *Sci. Am.* **181** [6] 22

Sperry, R. W. 1956. "The Eye and the Brain." *Sci. Am.* **194** [5] 48

FILMS

Flatworms (Platyhelminthes), 1962, (sd, b&w or c, 16 min), p or r, EBF

Reproduction, Growth and Development, Part V: *Regeneration*, No. 9, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Genetic Continuity

The background for genetics, the cellular basis of inheritance in sexual reproduction, although extremely important, is not represented by a student laboratory exercise. If possible, a film on meiosis should be shown and discussed. If this is not possible, demonstration (blackboard drawings) and discussion are necessary for further understanding of genetic mechanisms.

Do *NOT* locate genes on chromosomes, or even mention genes at this time, since their existence has not yet been demonstrated. Do not try to relate meiosis to heredity at this point. This may be done after the Mendelian laws have been discussed. In this discussion parallelism between the behavior of the postulated Mendelian factors (genes) and the observed behavior of chromosomes should be mentioned.

It is perhaps advisable to go through meiosis without chiasma formation the first time. Later it may be added, when the subject of crossing over is introduced.

The first two exercises are devoted respectively to *Drosophila* technique and to the concepts of randomness, chance, and probability. Both techniques and concepts need to be mastered in order to perform the exercises with *Drosophila* and in order to interpret adequately the results of the genetics exercises.

The next two exercises (30-3 and 30-4) are devoted to aspects of Mendelian genetics. Exercise 30-5 shows the influence of environment on heredity.

Exercises 31-1 and 31-2 deal with chromosomal aspects of heredity and introduce linkage, crossing over, and sex-linked inheritance. Here, chromosomal behavior should be pointedly correlated with meiosis.

Several of these exercises can be done using either *Drosophila* or Indian corn (maize). It is not intended that both materials be used in the same exercise, although they may be alternated from one exercise to another. You should use *Drosophila* if at all possible. The reasons for this recommendation are:

1. Interest on the part of the students is much greater because they learn new techniques (handling, etherizing, etc.), and because the changing counts from day to day make the outcome a bit uncertain.
2. Some of the experiments cannot be done with corn (no sex-linkage occurs in corn).

3. The questions arising with *Drosophila* are more numerous and penetrate to greater depths.

The nature of the action of genes is the subject of Exercises 32-1 and 32-2. The exercises for Chapter 33 encompass the area involving both genetics and evolution. Population genetics is the raw material of evolution and the exercise on human inheritance combines sex-linkage and population genetics.

Punnett squares (checkerboards) should not be used in the first analysis of a genetic cross. The Punnett square is simply a graphic, but laborious, way of finding an algebraic product. Since we want to emphasize the relation of mathematical reasoning and logic to genetics, we strongly recommend the algebraic method used in the textbook and in the exercises. This should not be difficult to accomplish with most classes, since the majority of students will be studying algebra at this time, and simple algebraic multiplication is one of the first procedures to which they are introduced. To fumble this opportunity to demonstrate the value and relevance of mathematics to its use in science is to throw away a valuable educational opportunity. Moreover, when linkage and crossing over are introduced, the checkerboards are no longer of value unless a coefficient is used for each row and column; this is also true of all the problems in the area of population genetics. In other words, the algebraic method can be used in all instances, whereas the checkerboard will not serve unless all the classes of gametes are equal in frequency – a very limited case.

In any discussion of human genetics the likelihood of apparent inconsistencies and perhaps some embarrassing situations must be anticipated. A common example is a brown-eyed child stating that both parents are blue-eyed. One should be prepared to meet such difficulties. It may even be desirable to stress a prime cause, oversimplification, *before* taking up a particular trait in class. Some common reasons for these apparent inconsistencies may be:

1. ***Oversimplification of the mode of inheritance.*** The case of eye color is a good example of this. All eyes are not either brown or blue, and furthermore there are various shades of each. More than one locus controls eye color and perhaps there are several or many alleles at a single locus which account for the various eye colors in man.
2. ***Misclassification.*** The range of phenotypes observable for a particular genotype is often considerable, and may lead to misclassification, especially when the range of the normal (or wild type) overlaps with the range of the mutant type. In the case of brown versus blue eye color in human beings, "blue" really means "absence of any pigment whatsoever in the front layer of the iris." The blue color itself is not a consequence of a pigment, but is a result of diffraction, like the blue color of the sky. The alternative phenotype is really, then, "presence of any pigment whatsoever in the front layer of the iris." Other gene loci determine the specific kinds of pigment that may be present, their relative amounts, etc. Obviously, a very small amount of pigment may be over-

looked and hence a "brown-eyed" person may be easily misclassified as "blue-eyed." It is not so easy to misclassify a blue-eyed person as brown-eyed; but it is the former misclassification that causes confusion.

3. *Adoption.*
4. ***Mutation.*** Cases in which one eye is blue and the other brown or in which a sector of the iris is different from the remainder are good examples of somatic mutations that are easily detected. Others are more difficult to establish as mutations.
5. *Illegitimacy.*

In the accompanying Schedule of Exercises for Genetics and Evolution, unshaded areas contain items which the teacher performs in advance in order to have *Drosophila* cultures ready on time. If the stocks are made up when suggested and kept in a 25° C incubator, there should be an abundance of virgin females on the day of the mating. Old cultures will yield very few virgins. To obtain virgins, completely empty the stock bottle of all adult flies *early* in the morning. All females collected during the next eight hours will be virgin. The late afternoon classes should have no trouble in obtaining virgin females, since the bottles will have been emptied over and over by earlier classes. If more virgin females are obtained than are needed by the early classes, they may be saved in vials for later classes. It is also desirable to put males into vials instead of discarding them while looking for virgin females, so that they will be available for matings. If it is necessary or seems desirable to have cultures of the stocks to compare with the offspring from crosses, a fresh stock bottle of each parent type should be made up at the time the cross is made.

It may be difficult to obtain enough virgin females for an 8:00 AM class unless the culture is taken home the night before. However, the culture must be kept as near 25° C as possible. The bottle can be emptied completely of all adult flies upon retiring, by 10 PM or later. All females separated from males in the morning by 9 or even 10 AM will then be virgin. Or, alternately, bottles may be emptied as soon as possible after arising, with the disadvantage however of losing the big "crop" of flies that emerge from the pupal stage in the very early morning hours. Another method of obtaining virgins is to put pupae in vials, one to a vial; but this is very laborious.

When the flies are etherized they may be moved about conveniently by a *very* small water-color brush. To move a large number from one place to another, a small spatula (cost less than 25 cents) is most satisfactory. The flies are brushed onto the spatula and then the spatula may be inserted into vial or bottle.

Vials and bottles into which etherized flies have been introduced should lie on their sides (with the medium at the bottom of the vial or bottle), so that the flies do not fall onto the medium. The containers should stay on their sides until all the flies have recovered from the ether and are walking or flying about. At this time the bottle may be set upright.

In using the suggested schedule, following, students should have a chance to deduce some of the basic Mendelian laws and concepts before reading about them. Since the procedure of obtaining laboratory experience and trying to reason from the data before knowing what to expect is a major objective of the BSCS, we strongly recommend that discussion of the results of the backcross (and perhaps prediction of the F_2) be carried out before the assignment on genetics in the textbook is read.

Do not be alarmed at the number of items per day listed in the schedule. Some of the items, such as "remove parents," take only one minute, though they are extremely important. It is *not* necessary to count flies every day; the flies may be allowed to accumulate for several days before being counted, but it is easier to handle smaller numbers at a time. It is important not to count beyond 10 days.

The schedule is only one of many possible schedules. Using it, you can plan and alter the schedule to suit the times available in your school. Only the times shown by shaded areas are essential for student performance in the laboratory. All other preparatory work can be done before or after class by the teacher, a student assistant, or assigned students. The original matings, for example, if done by the teacher at the appropriate time, will allow 8 class days to be saved by giving the students the F_1 generation to start with rather than the P_1 generation. It is important, however, that the student understand what you have done beforehand to supply him with the F_1 generation.

Another way to save time, room, and materials is for one team of students to do one experiment and others another, so that all counting for three experiments can be done simultaneously. It is important, however, for the data of all experiments to be given to all students. If this "leapfrogging" by student teams is done, it is imperative that all the results be discussed by the entire class.

Discussions and suggested times for Exercises 32-2, 33-1, and 33-2 may be shifted around considerably. It is felt that many discussions could come at the ends of laboratory periods, in which the first 15 minutes have been devoted to counting flies. This is especially easy if the flies are counted daily.

If a vacation week should cut into the schedule, someone will have to attend to the flies on at least one day during the vacation period. It is especially important by the 8th day to take the parents out of bottles in which crosses were made. In the event of a vacation this may be done as early as the 6th day (or if absolutely necessary by the 5th day) in order to consolidate work. Flies should be counted for the class on this day, or important data will be lost. If the counts are scheduled for the 10th or 11th day, they may be done on the 8th. This also makes it possible to start matings a day or two earlier than suggested in the schedule. In this case, however, be certain to move up the date for removal of parents.

Asbestos-center wire screen to prevent the medium from scorching while cooking. (Instead of the Pyrex flask, Bunsen burner, stand and screen, an ordinary sauce pan and a gas or electric hot plate may also be used. Stir with a wooden spatula while cooking the medium, and use a Pyrex beaker for filling the culture containers.)

Large Pyrex funnel with a short piece (6–8 inches) of rubber tubing attached and a burette or bulldog clamp for pinching the rubber tubing; or a glass or enamel pitcher with a fine lip, for pouring the melted food into bottles and vials

Strips of ordinary paper toweling, or doubled facial tissues (about 5 x 10 cm) for each stock culture bottle (much smaller strips should be used for vials). Some paper toweling is impregnated with insecticides. For this reason facial tissues may be used. Paper toweling or facial tissue should be cut, sterilized in a covered Pyrex dish or plugged milk bottle, and picked up with forceps when placed into the vial or bottle of sterilized medium

If facial tissues are used, fold them lengthwise so that they are about 5 cm wide and 22 cm long. Then cut in half. Each tissue makes strips for two bottles

Graduated cylinder, 100 ml or larger

Double pan balance

Wax pencil, marking tape, or labels

Filter paper (large) for weighing materials on the scale

Pressure cooker for sterilization

Measuring cup

Ring stand and clamp

Preparing the Medium

Many different kinds of media have been used for culturing *Drosophila.* It is generally agreed that all are suitable for proper development of the fruit flies if living yeast is added. Formulas for a wide variety of fermenting-media are detailed in the references listed at the end of this section. We recommend three formulas, one because of its simplicity of measurement and storage, one because of its economical use of agar and mold inhibitor, and one because of its dependability.

The first medium is a dry-mix food medium developed by W. F. Hollander, of Iowa State College. This mixture may be kept in dry storage until needed, at which time water is added to make up the desired amount. Add 5 to 6 ml of 0.5% propionic acid per liter of water, or some other mold inhibitor in the right proportion.

sugar	3 parts by volume
corn meal	2 parts by volume
brewers' yeast	1 part by volume
granulated agar	1 part by volume

All of the above ingredients are mixed thoroughly. Add about 1¾ cups of the dry mix to 1 liter of cold water, stirring it in carefully to prevent lumpiness as it is added. Boil gently for about 5 minutes or until the foaming stops. Transfer the mixture, after boiling, from the flask to the glass funnel mounted on the ring stand. Extend the rubber tube into the culture bottle or vial and regulate the flow of medium by using the clamp on the rubber tubing. Or pour the melted food into the fine-lipped pitcher from which it may be poured into bottles or vials. A steady hand is required to avoid getting food on the insides of the mouths of the culture vessels, where it will stick to the stoppers. Fill each bottle to a depth of 2.5 cm (1 inch) and each vial to a depth of about 2 cm. With a glass rod or a pencil, push one end of a doubled strip of paper toweling to the bottom of the medium while it is still soft. Plug the bottles with a cotton stopper wrapped with cheesecloth. (These stoppers may be used over and over if sterilized each time.) A fermentation vent to the bottom of the medium is sometimes needed when live yeasts are introduced; otherwise, CO_2 may build up rapidly under the medium and push it toward the top of the bottle or vial, trapping the flies. It may not be necessary to gouge a fermentation vent to the bottom of the medium if the paper strip is inserted deeply. The paper strips also serve the purpose of providing additional surface for egg laying and pupation. If facial tissues are used they should be placed in bottle just before putting in flies and not while the medium is still warm.

The second medium is a formula prepared by Bentley Glass at The Johns Hopkins University and is extremely economical in the use of agar, which is by far the most expensive single ingredient in any medium.

540 ml water
50 g corn meal
25 g rolled oats
50 g dextrose
5 g dry yeast
3 g agar
3 ml 0.5% propionic acid

The same procedure may be followed in preparing this medium as in the first medium. One should always estimate the amount of liquid medium needed to fill the culture containers, use an amount of water equal to this volume, and then add dry ingredients in proportion to the formula. One can figure that a half-pint bottle filled to a depth of 2.5 cm holds about 45 ml of medium; one liter of medium will therefore fill about 22 bottles, and the above formula will fill about 12 bottles.

The third formula is:

95 g corn meal
15 g agar
7.5 g dry yeast
75 ml molasses
75 ml corn syrup
850 ml water
5 ml 10% solution of *ortho*-phenylphenol (*o*-phenylphenol) in 95% alcohol

The *ortho*-phenylphenol (which can be purchased from Eastman Chemical Co.) is one of the most efficient mold inhibitors and has no noticeable effect on *Drosophila*. It can be substituted for the propionic acid in the above formulas. The *ortho*-phenol solution is not exactly 10%; it is made by dissolving 10 g of the powder in 100 ml of 95% ethyl alcohol. If there is a sediment, use only the supernatant fluid. Mix the above ingredients and cook to boiling with constant stirring to prevent sticking and formation of lumps. Fill bottles to a depth of about 3 cm. The above formula makes 18 to 20 bottles if filled properly. Vials should be filled about 3 cm. The above amounts may be halved but if much less than this is made at one time, too much water is lost in boiling and the medium is too dry.

Half-pint milk bottles are becoming scarce in some areas; other types of bottles will work just as well, if they taper toward the mouth and are wide-mouthed. It is still possible to purchase unmarked milk bottles in quantity from manufacturers who supply dairies. A local inquiry may identify a source of supply if you plan to use bottles in large numbers.

Sterilization

After the culture containers have been filled and plugged it is important to sterilize them in a pressure cooker or autoclave. The standard time of sterilization is 15 minutes at about 15 pounds of pressure. This procedure will usually kill bacteria and mold spores that are present in the medium. After sterilization, the culture containers may be stored in a deep freeze. If you have a freezer available, it may be possible to make up all the culture containers you need for a half year at one time. Simply allow the containers to thaw before yeasting.

Although vials and bottles may be stored in the deep freeze, it is not advisable to do so, since the medium contracts so much at very low temperatures that it pulls away from the walls of the container and is likely to fall when it is turned upside down while removing flies.

Inoculation with Live Yeast

After sterilization and cooling, and before use, inoculate 3 drops of a milky suspension of living yeast into each bottle or 1 to 2 drops in each vial. The dry, packaged yeast obtainable in grocery stores is quite satisfactory. The yeast should be allowed to grow for about 24 hours before the flies are introduced. If the media including brewers' yeast are used, inoculation with live yeast is not necessary, but may be helpful.

Securing Stock Cultures

Pure strains of *Drosophila* may be purchased from biological supply houses. In some areas stocks may be obtained from university biology departments if the demands are not excessive and adequate time is allowed for the filling of the request.

How to Start a *Drosophila* Culture

The flies you receive from stock centers will be in small vials. Use these to start your own stock cultures. Before you transfer the flies from the vials to your stock bottles, be sure there is no excess moisture on the food or on the sides of the bottles; flies can get stuck rather easily. Place a funnel in the new stock bottles. Before removing the cotton stopper from the vial, shake the flies to the bottom by tapping the vial on the table. When the flies have collected on the bottom of the vial, quickly remove the cotton stopper, invert the vial into the funnel, and transfer the flies to the fresh culture bottle by gently tapping the culture bottle on a rubber pad.

Do not discard the stock culture vial, but add a little yeast suspension and attach the vial to the side of the culture bottle with a rubber band. Eggs and larvae still in the vial will thus be saved to continue development and to provide more stock material.

Stock and experimental cultures should not be kept in the refrigerator, near a radiator, or on a window ledge in the sun. They should be kept in an incubator or constant temperature room at 25° C (77° F). The temperature of *Drosophila* cultures should not exceed 28° C (83° F) for any period of time, as the flies will then become sterile.

In the southern states it has been found that some species of small ants will crawl through the cotton plugs and chew up the developing flies. If this is likely to happen, we recommend that all culture bottles be placed on blocks in a pan of shallow water.

How to Maintain Stock Cultures

In making stock cultures, five to ten pairs of flies should be used as parents. Stocks should be changed once every 3 or 4 weeks to avoid contamination by mites (minute parasites that live on flies) and molds. Stocks should never be allowed to dry out, and water or yeast suspension should be added when moisture is required. At least two sets of cultures of each stock should be maintained in case one culture fails. Cultures can be changed on a rotating basis. It is important to remember that cultures should not be kept too long, and that old culture bottles should be *sterilized* and cleaned soon after they are no longer needed.

How to Collect Virgin Female *Drosophila*

Female *Drosophila* store the sperms which they receive during a mating, and use them to fertilize a large number of eggs over a period of time. It is therefore usually necessary that the females used in an experimental cross be virgin. Flies used in maintaining stock cultures need not be virgin, since the females could only have been impregnated by males from the same genetic stock.

Females usually do not mate within 10 hours after hatching. If *all* the adult flies are shaken from a culture bottle, the females which emerge during the next 10 hours are likely to be virgin when collected. Some geneticists recommend collecting the females after an 8-hour period. Virgin females may be collected over several periods of time and kept in a food vial. Each day two to three collections can be made from a culture bottle. Separate the males from the females and keep those you want to use. It is sometimes difficult to distinguish the sex of very young flies. Use the sex comb characteristic in males if you are in doubt. One stray male in a vial of (originally) virgin females will exclude the entire vial of flies from being used in an experiment. As a rule, put no more than 25 virgin females in a single vial.

How to Make a *Drosophila* Experimental Mating

When an experimental mating between virgin females and males of the desired genetic strains is made, the flies are put directly into a culture vial containing medium. If the flies are still etherized, place them on the side of the vial, which in turn should be placed on its side until the flies recover. A safe method for introducing etherized flies into a new vial is to invert the food vial over the etherized flies; they will crawl up to the food when they recover, and the cotton plug can then be inserted. In either case there should be no excess moisture in the new culture vial.

The parent flies in the experimental mating should breed, the female lay eggs, and the larvae develop to a good size within a week at room temperature. After 7 or 8 days, the parent flies should be shaken out of the culture vessel. This procedure is necessary in order to avoid confusing the parent flies with the offspring when the counts are made. Once flies begin to emerge they may be classified and counted for 10 days. After classifying and counting, the F_1 offspring may be discarded or put in other culture vessels for future use. Counting longer than this once again runs into the danger of overlapping generations, since the first F_1 flies may have mated before being counted, and hence have left some eggs to develop in the same culture vessel.

How to Keep Records of an Experiment

Adequate records are essential to the success of an experiment and an understanding of the results. Impress upon the student the importance of properly labeling both stock and experimental culture vessels, and of keeping a daily record of all observations relating to the experiments.

The wild-type fly is the standard phenotype from which all mutant types are inherited departures. Each mutant type is given a name suggesting the main diagnostic feature, preferably a simple, descriptive adjective. Names such as "white" (for white eyes, a recessive), or "bar" (for bar eyes, a dominant), are used.

Design a record sheet which will be adequate for your class, and have a number of them duplicated. A specimen record sheet, which could be adapted, is shown on page 202. The blanks after male parent and female parent should be filled with their respective phenotypes.

Anesthetizing and Examining *Drosophila*

In order to insure the effective development of skills in observing and handling *Drosophila* flies, we recommend that this equipment be made by interested students during their free hours or after school. The directions for each item of equipment are detailed below.

Etherizer. There are many types of etherizers in use and you may prefer one model over another. We recommend the following ones for ease of handling, simplicity of construction, and economy of cost.

Obtain the following items for each pair of students:

One shot glass of heavy construction; cost, 10 cents
One small aluminum funnel (6.5 cm or $2\frac{9}{16}$ inches); cost, 10 cents
One piece of heavy cotton string

With a pair of wire (diagonal) cutters, snip small notches about 0.5 cm apart around the entire bottom edge of the neck of the funnel (Fig. 30-1-1). Bend the small metal tabs at right angles to the neck of the funnel. Lay the string along the side of the neck with about 5 cm (2 inches) extending up along the outside of the neck of the funnel. Hold the funnel horizontally in your left hand (if you are right-handed) with the thumb securing the string. Now with your right hand begin to wrap the free end of the string around the funnel neck just above the beginning of the cone. Wrap the string in a single layer toward the end of the neck until the loose end of the string is held in place by the wrapping. When the tabs are reached, tie the two ends into a tight knot. Bend the tabs over the tightly wrapped string; this keeps the string from slipping off the funnel. The string will act as absorbent material for the ether, and the aluminum funnel will rest upon the top of the shot glass.

In the smaller community it may not always be possible to obtain the proper kind of shot glass in the local variety store. Most restaurant, hospital, or bar supply houses also have them.

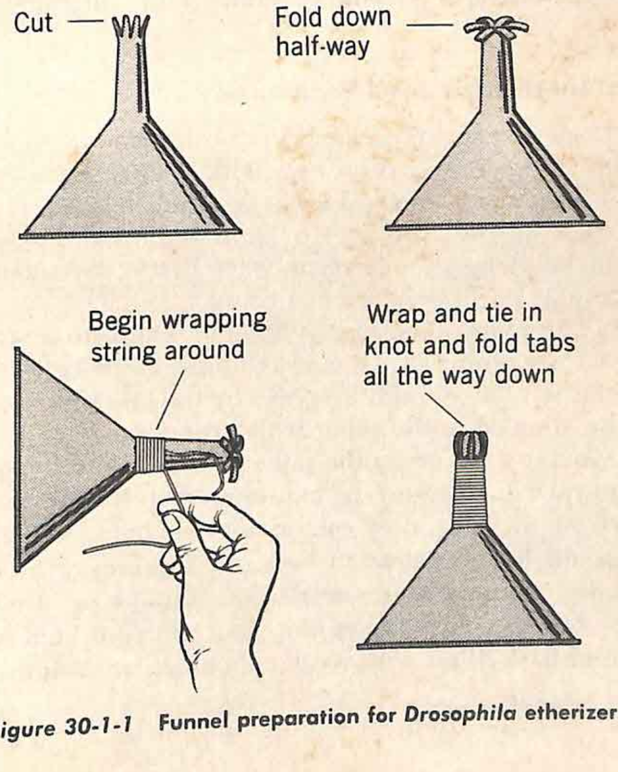

Cut
Fold down half-way
Begin wrapping string around
Wrap and tie in knot and fold tabs all the way down

Figure 30-1-1 **Funnel preparation for *Drosophila* etherizer.**

The common wide-mouthed bottle supplied with a cork, wire, and cotton tuft may be used as an etherizer, but it is not easily adapted to transfers of unetherized flies between containers whose mouth diameters are of different sizes (a bottle and a vial, for example). Several commercial etherizers on the market are satisfactory and can be purchased from biological supply houses. (The cost of a sufficient number of these etherizers to supply an entire class will be excessive for most budgets, however.)

Re-etherizer. A re-etherizer is not absolutely necessary but is desirable for use when students work slowly and some flies start to recover and walk away before all are classified and counted. In most cases the flies can simply be returned to the regular etherizer.

Materials needed for the construction of a re-etherizer are:

Top or bottom half of a glass Petri dish
Airplane glue
One piece of cotton gauze (bandage type), 2.5 x 7 cm (1 x 3 inches)

Fold the strip of gauze into a small compact pad of absorbent material. Use model airplane cement to attach the gauze pad to the inner bottom of the half Petri dish. Place a small drop of glue on each fold, and put a small weight on the pad to prevent its unfolding during drying. When the gauze has been stuck to the glass it can be lightly sprayed with ether and used to cover flies which are recovering from the anesthetic.

Morgue. For the morgue use any half-pint milk bottle, wide-mouthed jar, or baby food (screw-top) jar. Add 3 cm of motor oil and close with a cork or cap. Discarded flies are simply dumped into the morgue.

Procedure for Anesthetizing and Examining Flies

The ether (cheapest technical grade is quite adequate) should be distributed to the students in a dispenser. One dispenser of ether for each table should be adequate. Several kinds of ether dispensers can be used. For short periods of time, ordinary or ground-glass dropping bottles are suitable. A polyethylene squeeze-type wash bottle (4 oz, inert polyethylene, about 85 cents each) is very satisfactory.

CAUTION: The room should be ventilated when students are working with ether, and a warning of dangerous fumes emitted from such an explosive material as ether should be given by the teacher. No flames of any kind should be allowed in the room at this time.

The ether *must not* be left in the polyethylene container overnight, but should be returned to the storage can immediately after the school day.

After etherizing the flies, they can be dumped onto the tile for examination. Care should be taken not to overheat the flies with a strong light while examining them. Fluorescent lights should be used if the light source is to be brought close to the flies. Incandescent light can be filtered through a round flask filled with weak copper sulfate solution to filter out the heat.

If the flies are to be used for mating, do not leave them more than a minute in the etherizer. If they are to be counted and recorded only, they may be killed by being left for several minutes in the etherizer.

Lightly etherized flies will recover in about 5 minutes. If the flies should begin to wake up before the examination is completed they may also be re-etherized by covering them with the re-etherizer after the pad has been lightly sprayed with ether. In either case, a warning is needed that the flies are more easily killed by a second dose of ether than the first dose and should be anesthetized even more lightly.

Watch carefully to prevent the students from overetherizing their flies and killing them, thus limiting the supply of flies for the use of subsequent classes. If flies are overetherized, they turn up their wings vertically over their backs. If they react in this manner, place them in the morgue.

If flies are to be classified according to two pairs of alternative traits (four classes) they may first be separated into two piles on the basis of one pair of alternatives and then each of these piles separated into two on the basis of the second pair. Since the students will work slowly at first, it is best to place one of the two piles in a shell vial and to plug it with cotton. Then classify the other pile according to the second pair of traits. If the flies in the vial have awakened or started to move, re-etherize and then classify them.

Another method of recording is to put the pile of flies on one side of the field of the stereoscopic dissecting microscope, have one student move one fly into the center of the field, and call off the phenotype in full (that is, classify with respect to both pairs of traits) to a fellow student, who records. Then move the fly across to the other side of the field. This method is somewhat faster since each fly is inspected only once, but the classroom may be somewhat noisier because of many students calling out the phenotypes.

ANSWERS TO QUESTIONS

In the chart for recording sex differences in *Drosophila*, students will see that females are larger than the males; that the females have more bands on the abdomen and less of a dark tip; that females have a relatively larger abdomen; that sex combs are present on the front legs of the male; that the female abdomen is more pointed at its posterior end; and that external genitalia are more obvious in the male.

SUGGESTED READINGS

Books

Demerec, M. and B. P. Kaufman. 1957. Drosophila *Guide*. Carnegie Institute, Washington 5, D.C.

Morholt, E., P. Brandwein, and A. Joseph. 1958. *A Sourcebook for the Biological Sciences*. Harcourt, Brace & World, New York

Royle, H. A. 1957. *Laboratory Exercises in Genetics*. Burgess, Minneapolis

Articles

Hadhorn, E. 1962. "Fractionating the Fruit Fly." *Sci. Am.* **206** [4] 100

FILMS

Genetics Techniques: *Handling* Drosophila, 1960, (sd, c, 3 min), p or r, Thorne

Genetics, Part VI: *Genes and Chromosomes*, No. 5, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Demonstrates the preparation of a slide of the giant chromosomes from the salivary gland of the fruit fly larvae.

Genetics, Part VI: *Chromosomes and Sex*, No. 6, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

Deals with Morgan's experiments with *Drosophila.*

RECORD SHEET FOR *DROSOPHILA* CROSSES

Exercise No: ________

Male Parent: ____________ X Female Parent: ____________

Origin of Male Parent: ____________ Origin of Female Parent: ____________

Date of mating: ____________
Date of first larvae: ____________
Date of first pupae: ____________
Date of first adults: ____________

Dates of Counting	*Phenotypes*							
	1		2		3		4	
	♂	♀	♂	♀	♂	♀	♂	♀
Totals								
Grand Totals								

Analysis of the data:

RANDOMNESS, CHANCE, AND PROBABILITY

EXERCISE 30-2

TIME: One laboratory period plus homework assignment.

MATERIALS: See page 217 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on probability in Chapter 30 of the textbook.

■ **The purpose of this exercise is to demonstrate to the student the principles and operation of randomness, chance, and probability as related to genetic events.**

SPECIAL INSTRUCTIONS

Genetics exercises have, in general, a degree of difficulty for students because they deal with quantitative aspects of biology. Students with weak backgrounds in arithmetic and those who are not used to dealing with quantitative data frequently have difficulties with genetics exercises. Therefore, you should be sure to explain the basic arithmetic and algebra involved in the genetics exercises.

Every effort has been made to reduce arithmetic and algebra to a minimum. Nonetheless, this exercise affords a splendid opportunity to relate understandings in mathematics to understandings in biology, as all agree should be done. In this exercise, randomness, chance, and probability are dealt with by the simple expedient of throwing dice and observing the frequencies with which certain combinations occur. Then these, in turn, are translated into genetic terms.

Unless your students have had an adequate background in arithmetic and particularly in algebra, the expansion of the binomial should be omitted. Those students who have a background in algebra will find it an extremely helpful tool. Those who have not, still can be led to the same conclusions about randomness, chance, and probability, but by a more devious route.

This exercise is designed to be done in laboratory by individual students with a pooling of results. However, a sizable part of the exercise can be done as a homework assignment, as no elaborate equipment or supplies are required.

Students should have read enough material in Chapter 30 to be familiar with Mendel's experiments and such terms as F_1 and F_2 which are used in this exercise. If they have not, these terms will need to be explained.

TECHNIQUES

The dice used for this experiment should be of different colors or sizes, one color to stand for the egg, the other for the sperm, or a large die to stand for the egg, a smaller one for the sperm; or they may be temporarily marked to distinguish them.

Some teachers have preferred to use pennies rather than dice, and students can come to the same results by flipping one penny or two pennies and noting the number of times they come up heads or tails. However, as pennies are frequently dropped on the floor, or roll off the table, the use of dice has been found to be quieter and more convenient. Dice are commonly used in many children's and family games, but one must recognize that in certain communities there may be objections to their use in the classroom.

ANSWERS TO QUESTIONS

1. Six.
2. One in six.
3. Three.
4. One-half.
5. One-half.
6. Depends on student results, but the probability is only $({}^{252}/_{1024})^{10}$ or rather less than one in 4 million.
7. Again, depends on student results. The probability is ${}^{252}/_{1024}$, or slightly less than one in four.
8. It should be quite close to 50 : 50; closer than most of the results for 10 throws.
9. The percentage deviations for the small groups are larger, as a rule, than the deviation for the total of 100.
10. While the absolute deviation may be larger in the larger sample, the percentage of deviation is much smaller. Therefore, the larger the size of the sample, the smaller the percentage of deviation.
11. Yes, if expressed as a percentage. The size of the sample occasions a decrease in deviation which follows the law of diminishing returns, as indicated on the attached graph, Figure 30-2-1. This graph indicates roughly the relation between the sample size and percentage of deviation. It can be seen that, at first, an increase in the sample size is most effective in reducing the percentage of deviation and that, after the sample size has reached a certain magnitude, no further significant reduction takes place.
12. The increase in sample size from a small sample (10) to one ten times as large should lead to a large reduction in the percentage of deviation.
13. It must be large enough to reduce the percentage of deviation from expectation to a minimum. However, beyond a certain point an increase in size of the sample is not worthwhile, as can be seen in Figure 30-2-1.

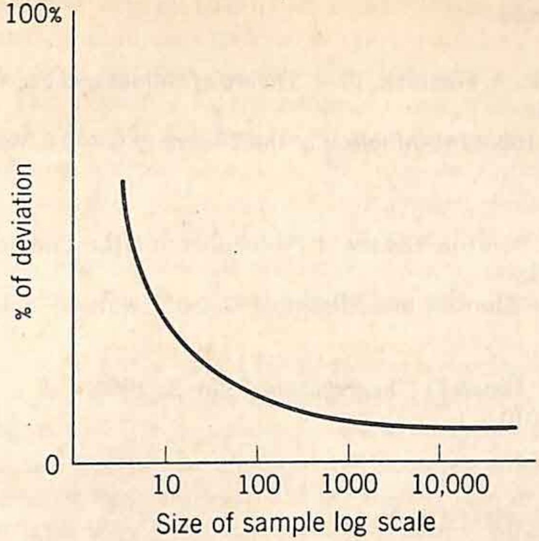


Figure 30-2-1 **Relationship of sample size and deviation.**

14. The results should approximate an equal frequency of each of the four combinations, each being approximately a quarter of the total.
15. The results for the entire class total will most likely provide a closer fit to expectation.
16. One-quarter.
17. It is the product of the probabilities. Sperm even = $1/2$; Egg even = $1/2$. Both even = $1/2 \times 1/2 = 1/4$.
18. This is a simple expansion of the binomial. $(1/2E + 1/2O) \times (1/2E + 1/2O) = 1/4EE + 1/4EO + 1/4OE + 1/4OO$.
19. This is the 1:2:1 ratio or $1/4 : 1/2 : 1/4$.
20. Both parents must be heterozygous.
21. 3 : 1, or three-quarters to one-quarter.
22. Three to one
23. $(1/2 : 1/2) \times (1)$
24. One parent would have to be heterozygous.
25. The other parent would have to be homozygous.
26. It will depend on the data. Usually there will be fairly good agreement if the total number of children exceeds 100.
27. One-quarter both boys; one-quarter boy-girl; one-quarter girl-boy; one-quarter both girls. Thus the possibility of having one of each sex is one-quarter plus one-quarter or one-half. The possibility of having children of one sex only is one-quarter plus one-quarter, or one-half. The ratio of families with two children of the same sex to families with two children of opposite sex is, then, one-half to one-half.
28. This will depend upon the collected data, but should approximate the expectation.
29. $(1/2)^3 = 1/8$.
30. $3/8$.

SUGGESTED READINGS

Books

Blackwell, D., and M. A. Girshick. 1954. *Theory of Games and Statistical Decisions.* Wiley, New York

McKinsey, J. C. Z. 1952. *Introduction to the Theory of Games.* McGraw-Hill, New York

Articles

Gardner, M. 1961. "On the Theory of Probability and the Practice of Gambling." *Sci. Am.* **205** [6] 150

Rapoport, A. 1962. "The Use and Misuse of Game Theories." *Sci. Am.* **207** [6] 108

FILMS

Genetics, Part VI: *Mendel's "Segregation,"* No. 2, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

INHERITANCE OF ONE-FACTOR DIFFERENCES

EXERCISE 30-3

TIME:

Alternative A: Matings begun by teacher 10 days prior to the exercise. One period for the exercise. Part of a period, 7 to 8 days later, to discard parental types. Period of counting for 8 to 10 days after the emergence of F_2 and backcross progeny.

Alternative B: One laboratory period.

MATERIALS: See page 221 in the *Student Laboratory Guide.*

This exercise is designed to accompany that section dealing with the inheritance of one-factor differences in Chapter 30 of the textbook and should follow class discussion of Mendel's one-factor crosses and conclusions.

■ **The purpose of this exercise is not to repeat Mendel's experiment with peas, but to verify the same principles through crosses, in Alternative A, between two different pure-breeding strains of *Drosophila* or, in Alternative B, between two different pure-breeding strains of maize.**

SPECIAL INSTRUCTIONS

As Alternatives A and B both demonstrate the inheritance of one-factor differences, but with different organisms, either Alternative A or Alternative B should be performed, depending upon the availability of time and materials, but not both.

For Alternative A the original mating should be made 10 days before the F_1 is to be examined, and 28 days before conclusion of the exercise. Eleven days before the original matings, stock cultures should be made up so that virgin females will be available for the original cross. If dumpy or vestigial wings are used as the mutant trait, use the mutant type as the female parent and the wild type as the male parent. If the reverse is done, the dumpy-winged or vestigial-winged male may have a hard time catching the normal-winged female to effect a mating. If the reciprocal mating is desired, the wild females' wings may be cut off with fine cuticle scissors. Do not cut the wings until they have hardened some hours after the flies have emerged from the pupal case.

For this exercise one cross might be between a virgin female dumpy-winged (wings truncated and reduced to about two-thirds the length of those of the wild type) and a wild-type male (red-eyed, gray-bodied, long-winged) and the reciprocal cross (dumpy male, wild-type female). Other *Drosophila* traits that are suitable for use in this cross are the recessive genes for sepia eye color (dark brown, almost black) or vestigial wings (reduced to stubs). Label the bottles according to the cross (*dp* females, +males) and add the date of the cross to the label. A + is the symbol used for wild type.

It is advisable to carry on more than one genetic experiment at a time. In order to accomplish this we recommend dividing the entire class into teams of two partners. Several teams can be assigned to one investigation while other teams simultaneously carry out different investigations. The reason for having several teams do the same experiment is a precautionary measure against possible contamination of one or two cultures and other errors. It also provides an opportunity for each team to check its results against those of the others, and thus learn something of the variability and replicability of a biological experiment. Moreover, a greater total number of offspring helps the student see that larger numbers of offspring are more likely to give results closer to the expected ratios.

In all exercises have partners decide among themselves who will be responsible for recording the information, making daily observations, assorting and counting, and other details. When all the investigations have been completed, the teams studying the same cross can pool their results, and select one or two individuals to report their procedures and findings to the whole class. Comparisons of similar investigations can be made between classes by tabulating results on the blackboard.

In order for your students to answer questions involving comparisons of their experimental results with theoretical expectations on the basis of some hypothesis (in this case the Mendelian expectation) they must know the chi-square method of determining the goodness of fit. This method is explained in the Appendix of the *Student Laboratory Guide*.

It may be desirable to finish the backcross and F_2 counts and discuss them before the students read the material on genetics in the textbook. Meiosis should have been discussed thoroughly before the students try to analyze the genetic results of crosses.

TECHNIQUES

Ears of corn showing 3:1 and 1:1 ratios can be obtained from any large biological supply house. They have the advantage of lasting for several years, providing you caution the students at the beginning of each class not to pick off the kernels and providing you store the ears in a tight container with naphthalene crystals to prevent insect damage.

Spraying the ears with a plastic spray will keep them clean, will make the removal of individual kernels more difficult, and will reduce damage by insects.

ANSWERS TO QUESTIONS

Alternative A.

1. The F_1 males are all alike in genotype. There has been no chance of the F_1 females crossing with any males but those needed to make the desired cross of F_1 females by F_1 males.
2. We need to record only males and females and the presence or absence of the mutant trait in question.
3. This will depend on the student results.
4. No. There is no blend.
5. Only one phenotype other than male and female.
6. Yes, in one.
7. In the backcross to the mutant type.
8. No. It is exactly the same.
9. The original wild type crossed with the mutant type gives only wild-type offspring. The F_1 wild type crossed with the mutant type gives $1/2$ wild and $1/2$ mutant in phenotype.
10. The offspring from the two matings just discussed are different.
11. The results indicate that the determiners of genetic traits are particulate—that is, that genes are inherited as units and that they are not divisible. Students may need help in arriving at the above conclusion. Note that we have no rigorous proof of this fact, but the mutant type has not blended with the wild type, become weaker, or broken up into subtypes. Also note that we have not established the existence of both dominant and recessive alleles, but only that there is some difference. For example, the recessive trait could be produced by lack of a gene rather than by presence of a different allele from the dominant. There is proof that recessive and dominant alleles are actually differences in DNA and not simply a presence and absence phenomenon, but such proof requires a study of multiple alleles and mutation.
12. Two.
13. The two kinds of backcross offspring should be approximately equal in number. Slight deviations from equality may be attributed to sampling error, and their significance can be checked by the chi-square method.
14. $1/2$.
15. $1/2$.

16. Recessive.
17. Because of the separation of homologous chromosomes, bearing the alleles *M* and *m*, in meiosis.
18. We would get a 4 : 4 : 1 genotypic ratio.
19. There would be only *MM* and *mm* offspring and a 50 : 50 ratio of dominant and recessive phenotypes.
20. This will depend on the class data.
21. Depends on student data, but most likely the results will not differ significantly from the expectation. A somewhat lower proportion of the mutant type is to be expected because mutants do not usually equal the wild type in viability.
22. **p** and **q** are equal.
23. It is random.
24. One backcross is the F_1 heterozygote crossed with the wild type; the other is the F_1 heterozygote crossed with the mutant type. In the first case only wild offspring would be observed; in the second, wild type and mutant would be in a 1 : 1 ratio.
25. A 1 : 1 ratio.
26. This will vary with class data, but will probably be close to the expected ratio.
27. Mendelian principles illustrated here are: (a) The principle of unit hereditary elements—that is, that inheritance is particulate. These particles are neither divisible nor dilutable. (b) The principle of segregation and recombination—that is, the pairs of genes for a single character separate (segregate) from one another during meiosis and are transmitted to the gametes; and sperms and eggs recombine at random at the time of fertilization, regardless of which alleles they may carry. (c) The principle of dominance, in which one gene of a pair can inhibit or mask the expression of the other member of that pair in the phenotype.

Alternative B.

1. In the F_2 ear the percentage will be 75%. In the backcross ear the percentage will be 50%.
2. In the F_2 ear the percentage is 25%; in the backcross ear it is 50%.
3. Yes. For the F_2 ear, 3 : 1. For the backcross ear, 1 : 1.
4. None. There is no blending or speckling.
5. It has had no effect. The recessive characteristic remains unmodified as it is transmitted.
6. Because when the backcross is made to the nonpurple type, both purple and nonpurple seeds are produced; but in the cross of pure purple by nonpurple, only purple seeds are produced.
7. They are particulate units which are not modified or blended.
8. They will show a 1 : 1 ratio.
9. If the proper ears are available, they should show this ratio.
10. 50%.
11. 50%.

12. This will depend on the students' results, but the deviations should be nonsignificant for most cases.
13. They have segregated as expected.
14. Homologous chromosomes separate, and so alleles segregate, in the process of meiosis.
15. There will be a 4 : 4 : 1 genotypic ratio or an 8 : 1 phenotypic ratio.
16. If fertilization were preferential there would be only *AA* and *aa* genotypes, and 50% of each of the two phenotypes.
17. This will depend on the class's data, but they should agree with the Mendelian expectation better than the data obtained by most individual teams.
18. This will depend on the students' results but they should not differ significantly from expectation.
19. That **p** and **q** are equal in number.
20. That fertilization is random.
21. See the answer to Question 27 in Alternative A.

SUGGESTED READINGS

Books

Goldstein, T. 1961. *Genetics Is Easy*. Viking Explorer Books, New York

Honegger-Lavater, W. and H. Burla. 1962. *Genetics, Heredity, Environment and Personality*. Dell, New York

Sinnott, E. W. *et al.* 1958. *Principles of Genetics*. 5th ed. McGraw-Hill, New York

Srb, A. M. and R. D. Owen. 1952. *General Genetics*. W. H. Freeman, San Francisco

Winchester, A. M. 1958. *Genetics*. 2nd ed. Houghton Mifflin, Cambridge

Articles

Dobzhansky, T. 1950. "Genetics." *Sci. Am.* **183** [3] 55

FILMS

Genetics, Part VI: *Mendel's "Segregation,"* No. 2, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Genetics, Part VI: *Mendel's "Recombination,"* No. 3, 1962, (sd, b&w, 29 min), p or r, AIBS(McGraw-Hill)

Heredity, 1938, (sd, b&w, 11 min), r, Indiana

Heredity, 1950, (fs, si, b&w, 67 fr), p or r, EBF

INDEPENDENT INHERITANCE

EXERCISE

30-4

TIME:

Alternative A: One laboratory period, plus a second laboratory period 10–12 days afterwards, and a third laboratory period 10–12 days after the second. (The time can be reduced to two laboratory periods 10–12 days apart, if the teacher makes the original cross.)

Alternative B: One laboratory period.

MATERIALS: See page 227 in the *Student Laboratory Guide*.

This exercise is designed to accompany the section on the dihybrid cross, in Chapter 30.

■ **The purpose of this exercise is to lead the students to understand the dihybrid cross by using either *Drosophila* or corn to demonstrate the results when two mutant types not governed by genes at the same location or even on the same chromosome are crossed.**

SPECIAL INSTRUCTIONS

Here again you have the opportunity of using either *Drosophila* or corn, this time to demonstrate the outcome of a dihybrid cross. Divide the class into four teams, each to make one of the crosses shown under Materials in the student guide. The time required for the *Drosophila* experiment may be reduced by making the initial crosses in advance and issuing to the students cultures in which the F_1 generation is beginning to emerge. In that event, students should be given flies of the dumpy and sepia types to compare with the wild-type flies, so that they may become acquainted with the traits which they are to examine and classify.

While Alternative B will prove useful as a substitute for Alternative A, in schools where there is a limited amount of equipment and space, or a limited amount of time, it presents the students only with the outcome of the genetic principle, and denies the students the opportunity to make crosses and see for themselves that the genetic materials operate according to specific predictable rules.

In Alternative A, the F_2 are expected to fall into four classes: wild type, dumpy nonsepia, nondumpy sepia, and dumpy sepia, in the ratio of $9/16 : 3/16 : 3/16 : 1/16$. The ratio of wild-type wing to dumpy wing is expected to be $3/4 : 1/4$; similarly, the ratio of wild-type eye to sepia eye is expected to be $3/4 : 1/4$. Both mutants have slightly reduced viabilities, so very possibly there will be some shortage of both dumpy and sepia. The double mutant, dumpy-sepia, shows even greater reduced viability, and this class may be considerably below expectancy, especially if the culture conditions are not optimal. A significant departure from expectation, as found by chi-square, means either that certain classes are subnormal in viability or that the expected ratio was based on the incorrect genetic hypothesis. All chi-square tells us is that the deviations are probably not due solely to chance. It does not tell us what is responsible or why.

TECHNIQUES

Ears of corn for Alternative B showing 9:3:3:1 ratios can be obtained from most biological supply houses. They have the advantage of lasting from year to year, provided that the students are cautioned not to pick off the kernels, and that the corn is stored in a tight container with naphthalene crystals or sprayed with an insect repellent. Spraying the ears with plastic sprays, as recommended under Exercise 30-3, Alternative B, is also worthwhile.

It must be made very clear to the student that he is dealing with independent factors which are not linked or bound to one another in any way. The custom of writing a dihybrid genotype as AASS or DDSS occasionally results in students believing that the characters are actually bound to one another in the chromosome. The teacher should emphasize the inheritance of these characteristics independently of each other and subsequent exercises on linkage will be far more meaningful.

ANSWERS TO QUESTIONS

Alternative A.

1. All the F_1 progeny are expected to be wild type.
2. Dumpy and sepia are both recessive.
3. Either *dpSe/Dpse* or *dpse/DpSe.*
4. The females need not be virgin because the males with which they might have mated previously are the same as for the desired cross.
5. The F_2 are expected to fall into four classes: wild type, dumpy nonsepia, nondumpy sepia, and dumpy sepia.
6. Four combinations, as mentioned in Answer 5 above.
7. The expected ratio of all normal-winged to all dumpy-winged individuals is 3 : 1, or, as we write it so that the students do not get the notion that there must be just four individuals in a monohybrid F_2, $^3/_4 : {}^1/_4$. Slight deviations from the expectations might be due to differential viability or to sampling error.
8. The expected ratio of red-eyed to sepia-eyed individuals is $^3/_4 : {}^1/_4$.
9. This will depend on student results, but there will probably be a significant difference, since the double mutant type will be rarer than expected and the wild type commoner. The deviations will be due to differential viability of the four types and to sampling error.
10. Nine.
11. All four phenotypes should have occurred in the F_2.
12. Wild type is expected to be the most common.
13. The double mutant type is expected to be rarest.
14. The single mutant types are about equally common.
15. It will depend on the data, but the poorer viability of mutant types and especially of the double mutant type may lead to a significant shortage of these types.
16. They should be alike.
17. The law of independent assortment may be stated as follows: "Alternative traits that depend on separate pairs of genes show independent assortment in passing from one generation to the next." In terms of the chromosome theory, we can say that they segregate independently in meiosis, and recombine independently in fertilization, and we must qualify the original law by adding, "*if they lie in different pairs of chromosomes.*"

Alternative B.

1. There are four phenotypes of kernels.
2. The most common are the purple smooth kernels.
3. The rarest are the nonpurple, shrunken ones.
4. The purple shrunken and nonpurple smooth are about equally common.
5. The F_1 generation could be written *AaSs* or *AaSS*.
6. No, because the F_1 ear could have been produced from either a cross *AASS* × *aass* or from a cross *AAss* × *aaSS*.
7. The types of sperms and eggs in the selfed organism will be the same genetically because meiosis produces the same results in segregation of the genes and independent assortment of different pairs of genes in both pollen grain and egg formation.
8. There are nine genotypes possible in the F_2.
9. It depends on the data, but usually there will not be a significant difference from the expected ratio.
10. The agreement should be better with the larger amount of data.
11. This indicates that an increase in sample size, up to a certain point, produces more reliable data.
12. The law of independent assortment (see the answer to Question 17 in Alternative A.)

SUGGESTED READINGS

Books

Sager, R. and F. J. Ryan. 1961. *Cell Heredity*. Wiley, New York

Sinnott, E. W., L. C. Dunn, and T. Dobzhansky. 1958. *Principles of Genetics*. McGraw-Hill, New York

Winchester, A. 1958. *Genetics*. Houghton Mifflin, Boston

Articles

Horowitz, N. H. 1956. "The Gene." *Sci. Am.* **195** [4] 78

FILMS

Principles of Genetics, No. 6: *Independent Segregation*, 1960, (sd, c, 29 min), p·or r, McGraw-Hill

See films for Exercise 30-3

HEREDITY AND ENVIRONMENT

EXERCISE
30-5

TIME: Part of an initial period for planting, and subsequent periodic observations over a span of approximately 2 weeks.

MATERIALS: See page 231 in the *Student Laboratory Guide*.

This exercise is designed to accompany the section dealing with the influence of environment on heredity in Chapter 30 of the textbook.

■ **The purpose of this exercise is to discover a way in which environment can modify the development of the inherited genetic potential of an organism.**

SPECIAL INSTRUCTIONS

Either tobacco or maize seeds may be used for this exercise. Tobacco seeds should begin to germinate in 7 to 9 days, and should have completed germination in another 3 days. In the uncovered dishes the ratio of green to albino seedlings should be three green to one albino. The covered seedlings will all be colorless until the covering is permanently removed. Then, those able to develop chlorophyll will develop it within 24 hours. There should then be an approximate proportion of three green to one albino.

Some of the better students will wonder how the recessive gene inhibiting chlorophyll development can be introduced into the heterozygous parents, since we might expect albinism in the homozygous recessive state to be lethal long before the plant can become reproductive. Actually the recessive gene only inhibits chlorophyll formation for a period of time. Many young albino plants ultimately form some chlorophyll and grow to maturity. They can thus be used as a source of the recessive gene. But generally the albino trait is carried in the heterozygous (nonalbino) plants, which can be crossed ($Aa \times Aa$) to prove that they are both carriers. All progenies from plants that yield any albino seedlings will then segregate in the 3:1 phenotypic ratio.

TECHNIQUES

Put two or three disks of filter or blotting paper in the bottom of each culture dish. This will reduce the chance of having the seeds or seedlings dry out.

Be sure to have students number the dishes according to some system so there can be no mistakes made in recording data. You might appoint one of the more dependable students to work out the class totals.

Tobacco seed, heterozygous for albinism, can be obtained from Carolina Biological Supply Co. Maize or sorghum, obtainable from the General Biological Supply House, Inc., shows the same kind of trait and yields the same ratio as does tobacco. If you use maize instead of tobacco, the seed should be planted in either vermiculite or topsoil, rather than spread on filter paper.

The table at the end of this exercise can be used as a model for recording the data. This table may be placed on the board for students to copy, or duplicated and given to the students.

ANSWERS TO QUESTIONS

1. Parents: $Aa \times Aa$
   Gametes: $(\frac{1}{2}A + \frac{1}{2}a)\ (\frac{1}{2}A + \frac{1}{2}a)$
   Offspring: $\frac{1}{4}AA$, $\frac{1}{2}Aa$, $\frac{1}{4}aa$
   Phenotype of offspring: $\frac{3}{4}$ green : $\frac{1}{4}$ albino
2. The differences observed are the result of genetic differences.
3. The difference would be attributable to heredity.
4. On the basis of class totals, the observed number of green and albino seedlings should be close to the expected ratio, $\frac{3}{4}$ green : $\frac{1}{4}$ albino.
5. If the dishes have been tightly covered, all of the seedlings should be albino.
6. The difference is attributed to an environmental condition, darkness, that has masked the effect of the dominant allele.
7. Without the presence of light (a factor of the environment), chlorophyll is not formed even if the genetic potentialities for its formation are present.
8. The percentages should be very close to those of the uncovered seeds. The percentage of green seedlings becomes much larger a day after the coverings are permanently removed.
9. In the presence of light, chlorophyll is formed in those individuals having the genetic potentiality to produce it; that is, in those seedlings carrying the dominant gene for chlorophyll formation.
10. Environmental and hereditary factors are responsible. There is an interaction between these factors in the development of the seedlings that were first covered and then uncovered.
11. The expression of a gene-controlled trait may depend upon suitable environmental conditions.
12. Both are important. It can be argued that the presence of the proper hereditary material is of greater relative importance, for without the hereditary potentiality for chlorophyll formation none would ever be formed no matter what the environment. Other students will make the logical point that without the necessary environmental conditions no chlorophyll would be formed regardless of the heredity.

SUGGESTED READINGS

Books

Dobzhansky, T. 1955. *Evolution, Genetics, and Man.* Wiley, New York

Hardin, G. 1961. *Biology.* W. H. Freeman, San Francisco
Chapter 35 deals with heredity and environment.

Articles

Hollander, W. F. 1952. "Lethal Heredity." *Sci. Am.* **187** [1] 58

FILMS

Heredity and Environment, 1959, (sd, b&w, 30 min), p or r, Indiana

Genetics, Part VI: *Nature and Nurture*, No. 9, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

DATA RECORD FOR HEREDITY AND ENVIRONMENT

	Albino	*Green*	*Percentage Albino*
Numbers of leaves in germinating tobacco or maize seeds (uncovered)			
8th day			
9th day			
10th day			
11th day			
12th day			
Numbers of leaves in germinating tobacco or maize seeds (covered)			
8th day			
9th day			
10th day			
11th day			
12th day			
Numbers of leaves in germinating tobacco or maize seeds (all dishes)			
12th day totals			

LINKAGE AND CROSSING OVER

EXERCISE 31-1

TIME:

Alternative A: One laboratory period, plus a second period 10–12 days later to collect and mate the F_1 generation, and a third period 10–12 days later for examination of the test cross progenies. (This time can be reduced if the teacher makes the original cross, and the students perform only the test cross.)

Alternative B: One laboratory period.

MATERIALS: See page 233 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on linkage and crossing over, in Chapter 31 of the textbook.

■ **The purpose of this exercise is to determine the patterns of inheritance when genes are linked together on the same chromosome, and to determine the recombinations which arise from crossing over.**

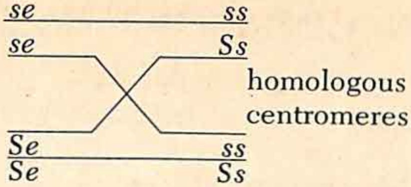
SPECIAL INSTRUCTIONS

Either Alternative A or B will demonstrate the principles of linkage and crossing over, and therefore, only one should be used.

For Alternative A, the mutants suggested are all recessive, so the F_1 is expected to be entirely wild type no matter how the cross is made. In the test-cross progeny it will make a great difference whether the mutants entered the F_1 from a single parent (coupled), or whether they entered from different parents (in repulsion). The two types of F_1 may be diagrammed as follows:

Coupling	**Repulsion**
se *ss*	*se* *Ss*
Se *Ss*	*Se* *ss*

where *ss* = spineless, *Ss* = nonspineless, *se* = sepia, *Se* = nonsepia. In the case of coupling, F_1 gametes of the parental types (*sess* and *SeSs*) are noncrossovers and more common; the crossovers, always the less common types, will be F_1 gametes carrying *seSs* or *Sess*. In the case of repulsion the common parental type gametes (F_1) will be the latter and the rarer types will be *sess* and *SeSs*, because these can be formed only by means of a crossover. The process of crossing over has been shown to involve an actual break in the homologous chromosomes at an identical level in the two, followed by the exchange of homologous segments. It may be depicted diagrammatically as follows:



This diagram takes account of the fact that crossing over actually takes place when there are four chromatids to a pair of chromosomes in the first division of meiosis. The crossover, at any single level, affects only two of the four strands.

For brown eye color and vestigial wings, both of which are also recessive mutants, use the symbols *bw* and *vg*, respectively. For nonbrown, use *Bw*; for nonvestigial, use *Vg*.

In *Drosophila* males there is never any crossing over, for reasons not known. Hence, when the F_1 fly tested is a male, there are only two classes in the test-cross progeny, namely, the same ones as the two parent (P_1) types.

The crossover frequency normally found between sepia and spineless is about 30%; between brown and vestigial, about 34%. The crossover values obtained for a given linkage, such as *seSs*, should be the same regardless of the coupling or repulsion type of the cross.

For Alternative B, students must be cautioned not to remove the kernels from the ear of corn. These ears can be kept indefinitely if they are stored in insect-proof containers with naphthalene crystals or sprayed with an insect repellent. The ears can be sprayed also with a clear plastic spray, which will hold the kernels firmly in place, keep the ears clean, and reduce insect damage.

TECHNIQUES

The size of student teams for Alternative A will depend on the size of the class and the number of cultures available as well as the amount of the materials and supplies.

The students are asked in Alternative A to record the types of offspring and their numbers on a data sheet as directed by the teacher. A chart suggested for recording test-cross progeny is given at the end of this exercise. It can be placed on the board for the students to copy in their notebooks, or duplicated and distributed to the class.

ANSWERS TO QUESTIONS

Alternative A.

1. Four classes, if F_1 females are test-crossed; two classes, if F_1 males are test-crossed.
2. No.
3. The two commoner classes will be wild type and double mutant in the crosses made by Teams 1 and 2. They will be the single mutant classes

(sepia nonspineless and nonsepia spineless) in the crosses made by Teams 3 and 4.

4. The product principle of probability would be applicable because of the independence of the two pairs of alleles. $(\frac{1}{2}Ss + \frac{1}{2}ss) \times (\frac{1}{2}Se + \frac{1}{2}se) = \frac{1}{4}Se\ Ss + \frac{1}{4}Se\ ss + \frac{1}{4}se\ Ss + \frac{1}{4}se\ ss$.
5. They should disagree, because of the linkage between the mutants.
6. Parental combinations.
7. Yes.
8. They should be approximately equal *but* the wild type will usually outnumber the double recessive (when they are expected to be equal) because of greater viability.
9. Same as for Question 8.
10. Answer should be yes. We can express the recombinations as a percentage of the total.
11. About 30% in the case of sepia and spineless; 34% in the case of brown and vestigial.
12. The values for a given linkage are expected to be the same when calculated from coupling or repulsion crosses.
13. The crossover value is determined by the distance between the two genes and not their distribution in the homologous chromosomes.
14. No crossing over occurs in male *Drosophila*.

Alternative B.

1. 1 : 1 : 1 : 1 : 1 : 1 : 1 : 1
2. This depends on the original cross. The parental combinations can be, for example, *C Sh Wx* and *c sh wx*, or *C sh wx* and *c Sh Wx*, or *C Sh wx* and *c sh Wx*, etc.
3. The percentage of recombination between *C* and *Sh* is roughly 3%; that between *Sh* and *Wx* is roughly 30%. Therefore, *Wx* and *Sh* have ten times as many recombinations as *C* and *Sh*.
4. The crossover values should be roughly comparable.
5. Yes.
6. Yes.
7. Approximately equal, but slightly smaller.
8. The map should indicate *C* at one end of the scale, *Sh* 6 mm to the side, and *Wx* 66 mm from *C* (60 mm from *Sh*).
9. The double crossovers do not get added into the recombinations between the terminal genes in the sequence.

SUGGESTED READINGS

Books

Bonner, D. M. 1961. *Heredity*. Prentice-Hall, Englewood Cliffs, N.J.
Snyder, L. H., and T. David. 1957. *The Principles of Heredity*. D. C. Heath, Boston.

FILMS

Principles of Genetics, No. 15: *Linkage*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Principles of Genetics, No. 16: *Crossing over in Terms of Meiosis*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Principles of Genetics, No. 17: *Crossing over, Chiasmata, and Genetic Maps*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Genetics, Part VI: *Chromosomes and Sex*, No. 6, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

Date ________

Exercise no. ______

CHART FOR RECORDING TEST-CROSS PROGENY

______♀______ × ______♂______

Date of Count	***Different Phenotypes of the Test-cross Progeny***			
Total				
Per cent				

SEX-LINKED INHERITANCE

EXERCISE

31-2

TIME: One period plus one period 10–12 days later, for the F_1 matings, and one period 10–12 days after the F_1 matings for the F_2 generation counts.

MATERIALS: See page 237 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on sex linkage in Chapter 31 of the textbook.

■ **The purpose of this exercise is to determine the type of inheritance that occurs when certain genes are carried on the sex chromosomes.**

SPECIAL INSTRUCTIONS

In this exercise the ratio of males to females and of red to white eyes in the F_1 generation is important, so the cross should be started by the students. It may be well to let different teams do the reciprocal crosses and compare their results. The total time required from start of the first cross to completion of the F_2 counts should be estimated at 4 weeks, and the very minimum is 23 days. The exercise must, therefore, be carried along with other work. The class will not have much to do except on the first day, the tenth and thirteenth days, and a few of the twenty-third to thirty-first days.

The cross of white-eyed females by red-eyed males produces red-eyed daughters and white-eyed sons. The reciprocal cross of red-eyed females by white-eyed males produces only red-eyed offspring and demonstrates that the white eye color is recessive. The problem, therefore, is to explain how you can have white-eyed male offspring in the first cross. This phenomenon is due to the fact that the Y chromosome carries no allele at the eye-color locus for white, thus allowing the recessive *w* to express itself when present in the X chromosome of a male. All F_1 females are heterozygous, X^WX^w, and, therefore, produce $\frac{1}{2}X^WY^-$ (red-eyed) sons and $\frac{1}{2}X^wY^-$ (white-eyed) sons, irrespective of what males they are crossed with. But the daughters of the F_1 females will all be red-eyed if the male parent is red-eyed, for they will be either X^WX^W or X^WX^w. If the male parent is white-eyed, X^wY^-, then half of the daughters (who must receive an X chromosome from the father) will be red-eyed and half will be white-eyed, for they will be equally X^WX^w and X^wX^w.

TECHNIQUES

The tables at the end of this exercise are suggested for reporting the data called for; F_1 progeny and F_2 progeny. A suggested format for the chart of sex-linked inheritance appears at the end of this exercise. This may be placed on the board for the students to copy in their notebooks, or duplicated and distributed to the students.

ANSWERS TO QUESTIONS

1. Red eye color is dominant.
2. In the F_1 progeny (red-eyed daughters and white-eyed sons), the red-eyed P_1 males did not contribute an allele for red eye color to their sons.
3. There is no allele of the eye color gene on the Y chromosome.
4. The female must be homozygous to have white eyes.
5. No, because the male offspring will always get their X chromosome

from their mother and will have no corresponding alleles on the Y chromosome.

6. It will matter in the female offspring, who receive one X chromosome from the mother and one from the father.

SUGGESTED READINGS

Books

Montagu, A. 1960. *Human Heredity.* Mentor Books, New York
Scheinfeld, A. 1950. *The New You and Heredity.* 2nd ed. Lippincott, Philadelphia
Stern, C. 1960. *Principles of Human Genetics.* W. H. Freeman, San Francisco

Articles

McKusick, V. 1962. "On the X Chromosome of Man." *Quart. Rev. of Biol.*, 37 June

FILMS

Principles of Genetics No. 12: *Sex-linked Inheritance*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill
Genetics, Part VI: *Chromosomes and Sex*, No. 6, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)
Genetics, Part VI: *Inheritance in Man*, No. 4, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

CHART FOR SEX-LINKED INHERITANCE

P_1
Genotype
Phenotype

Gametes

F_1
Genotype
Phenotype

Gametes

F_2
Genotype
Phenotype

F_1 PROGENY

P_1 ________ ♀ ________ × ________ ♂ ________

Date of Count	**Red-eyed**		**White-eyed**	
	♂	♀	♂	♀
Total				
Per cent				

F_2 PROGENY

F_1 ________ ♀ ________ × ________ ♂ ________

Date of Count	**Red-eyed**		**White-eyed**	
	♂	♀	♂	♀
Total				
Per cent				

GENETIC DIFFERENCES IN PEAS

EXERCISE

32-1

TIME: A portion of one period to prepare the peas for overnight soaking; a second period for observations and calculations.

MATERIALS: See page 239 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on the chemical nature of the gene in Chapter 32 of the textbook.

■ **The purpose of this exercise is to study round and wrinkled peas to show that differences that appear to be superficial and trivial may, in fact, probably be associated with biochemical (enzymatic) distinctions of great importance.**

SPECIAL INSTRUCTIONS

At the beginning of this exercise emphasize that the differences between round and wrinkled seeds cannot be attributed to the conditions of growth; for both round and wrinkled seeds may occur in the same pea pod, in any order from the stem end to the tip. A cross between pure round-seed and wrinkled-seed parents produces only round seeds; and a test cross of this F_1 hybrid by a pure wrinkled-seed plant produces round and wrinkled seeds in equal numbers. In the case of the pea, the seed, apart from the seed coat, contains only the embryo with its two cotyledons. The round and wrinkled traits of the seed are therefore the expression of the genotypes in the embryo offspring, and not of the maternal plant that produces the ovules.

Notice that the starch test for the soaked peas requires two experiments of 30 minutes each. These consecutive experiments cannot be performed within the time allotted for a normal laboratory period; therefore the first part of this starch test should be begun some 20 minutes before the laboratory period is to begin, in order for the students to see the results of the first test and to perform the second one within the allotted time.

It may be possible to assign the starch test as a special project to be done by a limited number of students and demonstrated to the class.

TECHNIQUES

Each student should be supplied with 25 dried round peas, 25 dried wrinkled peas, and two small bottles to hold the peas.

Glucose-1-phosphate can be bought from chemical supply companies at about $1.00 per gram. One gram is enough for 20 small Petri dishes. If you use small dishes and thin layers of agar, the cost will be kept down. Cover the dishes and keep them in the refrigerator until an hour before class time. If they are not warmed up sufficiently, the reactions may be too slow for the students to observe. You will get the best results if the plates are placed in an incubator at 37° C for 30 minutes after the extract has been placed on them.

A Waring blendor can be used in place of mortar and pestle. After preliminary straining, a centrifuge will remove the coarse starch grains from the extract and eliminate the necessity of blotting the spots for the starch test.

A homogeneous blue spot will usually appear, indicating where starch is synthesized in the agar. The 30-minute test shows a blue ring rather than a solid spot.

The starch-forming enzyme is starch phosphorylase, which joins the glucose molecules together to form unbranched chains, making up the amylose fraction of natural starch. The other fraction of starch is amylopectin, whose synthesis needs another enzyme, one that is unstable under the conditions of this exercise.

You will find suggested forms for tables on which to record the data at the end of this exercise. They may be put on the board or duplicated and distributed to the class.

ANSWERS TO QUESTIONS

1. The wrinkled peas (which are not wrinkled until they have been dried) absorb more water than the round peas.
2. The grains of starch from round peas are simple and oval in shape, and look rather like a lot of Irish potatoes of different sizes. The starch grains from wrinkled seeds are round in shape and compound, being subdivided into quadrants or possessing grooves.
3. The preparations of other students should be essentially the same.
4. Because the cover can be removed and accidentally placed on a different dish, or it may be rotated and become reversed from its original position on the dish.
5. The enzyme is stronger in the extract from wrinkled peas.
6. Genetic differences.
7. The biochemical level.
8. A gene controls the development of a structure by doing something chemical; therefore, the chemical aspect of gene action is basic.

SUGGESTED READINGS

Books

Strauss, B. 1960. *An Outline of Chemical Genetics.* W. B. Saunders, Philadelphia

Articles

Bearn, A. G., and J. L. German. 1961. "Chromosomes and Disease." *Sci. Am.* **205** [5] 66

Bearn, A. G. 1956. "The Chemistry of Hereditary Disease." *Sci. Am.* 195 [6] 126
Mirsky, A. E. 1953. "The Chemistry of Heredity." *Sci. Am.* 188 [2] 47

FILMS

Principles of Genetics, No. 39: *Biochemical Genetics I*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill
Principles of Genetics, No. 40: *Biochemical Genetics II*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill
Principles of Genetics, No. 41: *Gene Structure and Gene Action*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill
Principles of Genetics, No. 42: *Genetic Activity and Chromosome Chemistry*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill
Genetics, Part VI: *Biochemical Genetics*, No. 11, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

DATA SHEET FOR GENETIC DIFFERENCES IN PEAS

1. Weight of rinsed *round-peas* bottle + dry *round peas* ____ . ____g
2. Weight of rinsed *round-peas* bottle ____ . ____g
3. Weight of 25 *round peas* (Calculate) ____ . ____g

4. Weight of rinsed *wrinkled-peas* bottle + dry *wrinkled peas* ____ . ____g
5. Weight of rinsed *wrinkled-peas* bottle ____ . ____g
6. Weight of 25 dry *wrinkled peas* (Calculate) ____ . ____g

7. Weight of *round-peas* bottle + soaked *round peas* ____ . ____g
8. Weight of rinsed *round-peas* bottle (from 2 above) ____ . ____g
9. Weight of 25 soaked *round peas* (Calculate) ____ . ____g

10. Weight of *wrinkled-peas* bottle + soaked *wrinkled peas* ____ . ____g
11. Weight of rinsed *wrinkled-peas* bottle (from 5 above) ____ . ____g
12. Weight of 25 soaked *wrinkled peas* (Calculate) ____ . ____g

CALCULATIONS

Weight of soaked *round peas* (number 9) ____.____g

Weight of dry *round peas* (number 3) ____.____g

Weight difference ____.____g

$$\frac{\text{weight difference (}\qquad\text{g)}}{\text{dry weight (}\qquad\text{g)}} \times 100 = \_\_\_\_\_\%$$ increase of *round peas*

Weight of soaked *wrinkled peas* (number 12) ____.____g

Weight of dry *wrinkled peas* (number 6) ____.____g

Weight difference ____.____g

$$\frac{\text{weight difference (}\qquad\text{g)}}{\text{dry weight (}\qquad\text{g)}} \times 100 = \_\_\_\_\_\%$$ increase of *wrinkled peas*

ENZYME EXTRACT OF ROUND PEAS

Drop	*Time (Minutes)*	*Appearance*	*Assumption*
1.	30		
2.	30		
3.	60		
4.	60		

ENZYME EXTRACT OF WRINKLED PEAS

Drop	*Time (Minutes)*	*Appearance*	*Assumption*
1.	30		
2.	30		
3.	60		
4.	60		

NUTRITIONAL MUTANTS IN *NEUROSPORA*

EXERCISE
32-2

TIME: One period, or as homework.

MATERIALS: None

This exercise is designed to accompany the section on *Neurospora*, in Chapter 32 of the textbook.

■ **The purpose of this exercise is to trace the experimental steps that led Beadle and Tatum to their Nobel prize-winning discovery of the primary effect of a gene.**

SPECIAL INSTRUCTIONS

This exercise is designed to give the requisite information to all students, and to ask challenging questions of the best students. It will prove to be difficult for most students.

TECHNIQUES

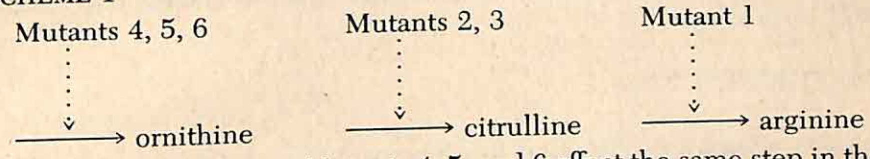
Since this exercise can be done without work in the laboratory, it can be assigned for any convenient time, either as homework or as an exercise to do between parts of the genetics exercises being performed in the laboratory.

ANSWERS TO QUESTIONS

1. Those forms having no growth have mutations that make utilization of the minimal medium impossible.
2. Those forms that do not grow on the minimal medium must require some additional nutritional materials.
3. The spores are first cultured on a complete medium so that even mutant types with biochemical deficiencies can grow; later testing on the minimal medium reveals the presence of defects.
4. Mutations must have made it impossible for the mutant molds to synthesize one or more vitamins, which are lacking in the minimal medium. The wild type can carry on these syntheses.
5. The second type of mutations must have caused a deficiency in the ability to synthesize one or more amino acids, since these mutants will grow only when amino acids are added to the minimal medium.
6. This mutation has made it impossible for the organism to synthesize arginine.

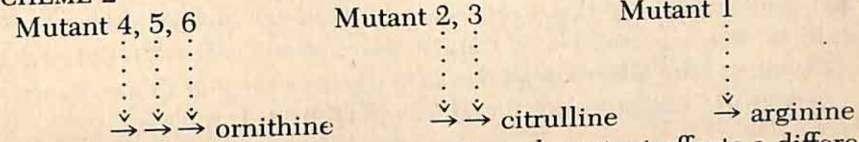
7. No, Strain 1 cannot grow on minimal medium.
8. Yes, Strain 1 can grow on minimal + arginine.
9. No, neither ornithine nor citrulline can take the place of arginine for Strain 1.
10. No, Strain 2 cannot grow on minimal medium.
11. Yes, Strain 2 can grow on minimal + arginine.
12. Yes, citrulline can replace arginine as a supplement to minimal medium for Strain 2.
13. The most logical assumption is that Strain 2 converts citrulline to arginine.
14. The mutation in Strain 2 must affect a step in the synthesis that comes before the one affected by mutation in Strain 1. The reasoning is that the step blocked by mutation in Strain 2 can be bypassed by supplying either citrulline or arginine, whereas supplying citrulline does nothing for Strain 1 since the blocked step comes after the production of citrulline.
15. Two possible schemes are:

SCHEME 1



In the above scheme, Mutants 4, 5, and 6 affect the same step in the synthesis, thus 4, 5, and 6 could be alleles of each other.

SCHEME 2



However, as is shown in scheme 2, each mutant affects a different step in the synthesis of ornithine; thus Mutants 4, 5, and 6 are not considered to be alleles in this scheme.

Notice that in either of the two schemes the order is ornithine → citrulline → arginine.

SUGGESTED READINGS

Books

Beadle, G. W. and E. L. Tatum. 1941. "Genetic Control of Biochemical Reactions in *Neurospora*." in M. L. Gabriel and S. Fogel. 1955. *Great Experiments in Biology*, p. 273. Prentice-Hall, Englewood Cliffs, N. J.

Horowitz, N. H. 1960. *Chemical Genetics*. California Institute of Technology, Division of Biology, Pasadena, Calif.
A laboratory syllabus on *Neurospora*.

Moore, R. 1961. *The Coil of Life*. Knopf, New York

Articles

Beadle, G. W. 1948. "The Genes of Men and Molds." *Sci. Am.* **179** [3] 30

FILMS

Principles of Genetics, No. 39: *Biochemical Genetics I*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill.

Principles of Genetics, No. 40: *Biochemical Genetics II*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill.

Genetics, Part VI: *Biochemical Genetics*, No. 11, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill).

A POPULATION GENETICS STUDY

EXERCISE 33-1

TIME: Whatever necessary for gathering data from family and neighbors, and for analyzing it.

MATERIALS: None

This exercise is designed to accompany the section on population genetics in Chapter 33 of the textbook.

■ **The purpose of this exercise is to discover how common a given trait is in a representative human population, and what are the frequencies of the alternate alleles in the population for this trait, according to the assumptions of the Hardy-Weinberg Principle.**

SPECIAL INSTRUCTIONS

This exercise can be assigned as homework to be done outside of class time, with the results to be discussed during the intervals between experimental portions of other genetics exercises.

This exercise is extremely simple in procedure, but involves more and more sophisticated thinking in mathematics as it proceeds. Accordingly, it is suggested that all the students take part in gathering data and in class analysis of the pooled data. Procedure D is fundamental to the interpretation of population genetics data, and should be required of all students with sufficient mathematical ability to carry out the calculations. It may be emphasized that these calculations and interpretations are far simpler than many the students are expected to perform in first-year algebra. Procedure E has particular interest because it enables the student to predict frequencies in the population of various types of matings. The student can calculate the probability that a given mating, involving parents both of whom are of the dominant type, will be between two heterozygotes, and hence may produce the recessive type of offspring; or the probability that a mating involving one parent of the dominant type and one of the recessive type will involve a heterozygous dominant person, and therefore may produce the recessive type of offspring.

Tongue rolling is thought to be inherited as a simple dominant trait, but do not inform the students of this. Let them discover it from their own family data collected by the class, which should be adequate for the purpose.

When students are gathering data from neighbors, they should be cautioned to approach only those neighbors living in their immediate vicinity and who know them. Assigning students, even in their own neighborhoods, to approach strangers, can lead to difficulties.

As a public-relations gesture while collecting data in the neighborhood, students should be prepared to give intelligent answers on why the data are being collected, and for what purpose they are to be used.

In place of, or in addition to tongue rolling, the ability to taste phenylthiocarbamide (PTC), can be used for this exercise. The techniques involved are outlined on pages 191–92 of Morholt, *et. al.* listed under Suggested Readings at the end of this exercise.

TECHNIQUES

For extracting the square root of the percentage of the recessive trait in the population in order to derive the frequency of the recessive allele in the population, a table of square roots may be provided. If not, remember to emphasize that the square root of a fraction, decimal, or percentage, is always *larger* than the original value. It is helpful also to keep in mind the square roots of the percentages that are simple numbers:

The square root of 0.01 is 0.1
0.04 0.2
0.09 0.3
0.16 0.4
0.25 0.5
0.36 0.6
0.49 0.7
0.64 0.8
0.81 0.9

ANSWERS TO QUESTIONS

1. The percentage of tongue rollers in the family data collected will be rather different from that of the class itself, or from the random sample of the population. But, except for the factor of chance, which will make the smaller sample (the class) more subject to unpredictable variation than the larger sample (the population), the class percentage

of tongue rollers and the percentage in the general sample should agree quite well. Since they are both random samples, they may be pooled.

2. Tongue rolling is apparently dominant.
3. A single family in which both parents are tongue rollers, but one child or more is not, is sufficient to prove that tongue rolling is not recessive. But a family composed of two tongue-roller parents and only tongue rolling children does not necessarily prove the point. If either parent has the homozygous dominant genotype, the recessive trait will never show up in the children. In families where one parent is a tongue roller and the other is not and where the offspring express both phenotypes, the expectation among the offspring is ½ tongue rollers, ½ nonrollers. But such crosses do not inform you which alternative trait is dominant. If tongue rolling were a simple recessive trait, the mating of a tongue roller by a tongue roller should produce only tongue-roller children. But this is not a critical test, since the cross of dominant by dominant can also be restricted to one type of offspring.
4. The answer will depend on the student data, but the results should be consistent with the data from the class itself.
5. The frequency of the recessive allele in the population is always greater than the frequency of the recessive trait.
6. The answer to this will depend on the data.
7. The ratio in the population of dominant-type persons to recessive-type persons for any particular trait has no relation to the ¾ : ¼ Mendelian expectation, which applies only to a particular kind of mating. This is perhaps the most important difference to recognize in population genetics.
8. It scarcely seems likely that human marriages are in any way influenced by the ability to tongue roll or its lack.
9. The chosen example of eye-color frequencies is a typical Hardy-Weinberg distribution, where $\mathbf{p} = 0.70$ and $\mathbf{q} = 0.30$.
10. The frequency of matings of a tongue roller by a nontongue-roller is $2(\mathbf{p}^2 + 2\mathbf{pq})\mathbf{q}^2$. A mating of the heterozygous type with the homozygous recessive type would have the frequency of $2(2\mathbf{pq} \times \mathbf{q}^2)$, or $4\mathbf{pq}^3$. This is the simplest sort of algebra, and represents a fine opportunity to show students that algebra has actual usefulness in the field of biology.

SUGGESTED READINGS

Books

Morholt, E., P. Brandwein, and A. Joseph. 1958. *A Sourcebook for the Biological Sciences.* Harcourt, Brace & World, New York

Stern, C. 1960. *Principles of Genetics*, 2nd ed. W. H. Freeman, San Francisco
Contains a good discussion of the Hardy-Weinberg Principle.

Wallace, B. and A. M. Srb. 1961. *Adaptation.* Prentice-Hall, Englewood Cliffs, N.J.
Considers genetics as related to the problems of adaptation.

FILMS

Principles of Genetics, No. 24: *Genetics of Mendelian Populations*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Principles of Genetics, No. 25: *Genetic Loads in Mendelian Populations*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Genetics, Part VI: *Inheritance in Man*, No. 4, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

Genetics, Part VI: *The Treasury of Human Genes*, No. 12, 1962, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

HUMAN INHERITANCE

EXERCISE 33-2

TIME: Can be used as a homework exercise with the results pooled and discussed in class between laboratory work on other genetics exercises.

MATERIALS: None

This exercise is designed to accompany the section on human inheritance, sex linkage, and population genetics, in Chapter 33 of the textbook.

■ **The purpose of this exercise is to determine how sex-linked factors are transmitted in a single family and then to determine the frequency of the alternate alleles in the general human population.**

SPECIAL INSTRUCTIONS

This exercise combines the sex linkage of Exercise 31-2, with the population genetics approach of Exercise 33-1.

While the mathematics in this exercise is simpler than that which most students have had in a beginning algebra class, you may find it necessary to reinforce their learning with a short review of the mathematics involved.

The pedigree aspect of genetics must be handled with extreme caution. The teacher cannot be expected to know the family backgrounds of the students, but should tread warily lest a student be embarrassed. A student may commit himself in front of the class before realizing that adoptions and remarriages affect the construction of a correct genealogical chart.

TECHNIQUES

The two tables at the end of this exercise will be useful as a pattern for student records in this exercise. You may wish to place them on the board for the students to copy, or duplicate and distribute them to the class.

ANSWERS TO QUESTIONS

1. Recessive.
2. It is sex linked.
3. The color-blind individuals in families shown in Pedigrees I and II include three sons, neither of whose parents are color-blind. The allele for color blindness consequently must be recessive. All the sons of color-blind women are color-blind. Therefore, color-blind males always inherit the trait through their mothers. Fathers of all color-blind girls are color-blind. If the mother is not color-blind, but one of her sons is, ½ of her daughters are carriers and ½ of her sons are color-blind. These are all evidences that the allele for color blindness is sex linked.
4. All the males have normal color vision.
5. None is color-blind.
6. All have normal color vision.
7. All are carriers.
8. None is color-blind.
9. One-half the males have normal color vision.
10. One-half the males are color-blind.
11. All the females have normal color vision.
12. One-half the females are carriers.
13. None is color-blind.
14. The hemophilic carriers in the royal pedigree include Queen Victoria herself, her daughter Alice of Hesse, her daughter Beatrice of Battenberg, her granddaughter Irene of Prussia, her granddaughter Alexandra of Russia, her granddaughter Alice, and her granddaughter Victoria Eugenie of Spain.
15. Her father was either color-blind (Hc) or hemophilic (hC).
16. That he was color-blind is far more probable. About 8–10% of all European males are color-blind; very few hemophilic males reach adulthood and produce children.
17. Four kinds. Noncrossovers: color-blind Hc, or hemophilic hC; crossovers: normal vision nonhemophilic HC, and both color-blind and hemophilic hc.
18. For noncrossovers, the probability is 0.90/2, or 45% for each type. For crossovers, 0.10/2, or 5% for each type.
19. Noncrossovers: Hc/HC and hC/HC. Crossovers: HC/HC and hc/HC. All normal for color vision and all nonhemophilic.
20. Depends on student data. Probably around 8%.
21. Depends on student data; more likely to be closer to 8% than the smaller sample represented by the class.
22. Depends on student data. Probably less than 1%.
23. Depends on student data. As above, less than 1%.
24. In the United States approximately 8% of the men are color-blind. Since allele frequency is the same as genotype frequency for sex-linked genes in the male, $\mathbf{q} = 0.08$ and $\mathbf{p} = 0.92$. If mating has been at

random, then **p** and **q** in males and females are the same, and $\mathbf{q}^2$ is the expected frequency in females ($0.08 \times 0.08 = 0.0064$).

25. $\mathbf{p}^2 = 84.64\%$.

26. $2\mathbf{pq} = 14.72\%$.

27. $\mathbf{q}^2 = 0.64\%$.

28. Depends on the student data. $2\mathbf{pq} = ?$

SUGGESTED READINGS

Books

Goldstein, P. 1961. *Genetics is Easy*. Viking Explorer Books, New York

Scheinfeld, A. 1950. *The New You and Heredity*. Lippincott, Philadelphia

Stern, C. 1960. *Principles of Human Genetics*. W. H. Freeman, San Francisco

Articles

Bearn, A. G. and J. L. German. 1961. "Chromosomes and Disease." *Sci. Am.* **205** [5] 66

FILMS

Principles of Genetics, No. 12: *Sex-linked Inheritance*, 1960, (sd, b&w, 29 min), p or r, McGraw-Hill

Genetics, Part VI: *Inheritance in Man*, No. 4, 1962, (sd, c, 29 min), p or r, AIBS (McGraw-Hill)

COLOR BLINDNESS IN CLASS STUDY

	Boys	*Girls*
Color-blind		
Normal color vision		

COLOR BLINDNESS IN GENERAL POPULATION STUDY

	Boys and Men	*Girls and Women*
Color-blind		
Normal color vision		
Total		

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Evolution

Evolution exercises actively involving the students in laboratory or field work are difficult if not impossible to plan for students at this level because of limited time and limited facilities in high schools.

Therefore, four of the five exercises are essentially exercises in scientific thinking and interpretation of data, and only Exercise 36-2 requires laboratory facilities. The other four can be appropriately fitted into the class schedule or assigned as homework exercises, whichever is desirable.

The first two exercises (34-1 and 34-2) involve natural selection. The first shows selection operating to alter allele frequencies in two different environments, the second involves the selection of the heterozygote in human populations.

Exercise 36-1 involves an examination of adaptation, and Exercise 36-2 introduces the student to fossils as evidence of evolution.

SELECTION AND ALLELE FREQUENCIES

EXERCISE

34-1

TIME: A homework exercise to be discussed in class.

MATERIALS: None

This exercise is designed to accompany the sections on Darwin's conception of natural selection and adaptation and selection in Chapter 34 of the textbook.

■ **The purpose of this exercise is to examine the frequencies of two alleles maintained in a population: first, when completely isolated from disturbing influences; and second, when under the pressure of natural selection favoring one type more than the other.**

SPECIAL INSTRUCTIONS

Since this exercise requires no special materials or equipment, it can be assigned as homework to be discussed later in class. It can be used in conjunction with the exercises in genetics during the times when laboratory work is held in abeyance awaiting subsequent generations of fruit flies.

In Part A, Gene Frequencies in Populations, we try to banish the common but erroneous idea that was of great concern to Charles Darwin himself, that hereditary variations in a population tend to disappear by merging and blending and destroying their opposites. W. S. Laughlin of the University of Wisconsin has termed this imaginary process "genophagy," in which dominant genes somehow eat or destroy recessive genes in a population. The most familiar form of this superstition is the idea that blood is blended in hybrid individuals.

In Part B, Natural Selection, you may want to discuss the splitting of the original moth population into two distinct subpopulations before the class undertakes to answer the questions.

TECHNIQUES

Review with the students the 1908 work of Hardy and Weinberg, wherein each man independently realized that both the allelic and the genotypic frequencies in a randomly breeding population remain constant from one generation to the next in the absence of disrupting factors such as mutation, selection, and immigration.

In this exercise, selection serves as a disrupting factor.

ANSWERS TO QUESTIONS

1. The chances of mating of a black male with a female of either color will be equal because there are equal numbers of the two kinds of females.
2. The same answer as for Question 1 above.
3. *WW* ♀ × *WW* ♂; *WW* ♀ × *ww* ♂;
   ww ♀ × *WW* ♂; *ww* ♀ × *ww* ♂.
4. No, each cross produces only one kind of offspring.
5. The proportions of black and speckled white moths in the offspring population will be 75% black and 25% speckled white.
6. No, it is obviously different from that which existed at the start.
7. 8. and 9. The percentages of *W* and *w* remain 50% for each.
10. The frequencies of *W* and *w* remain the same (0.5), in both the parent and offspring populations.
11. When one starts with 0.40 *WW* and 0.60 *ww* moths, the gametes are in the frequency of 0.40 *W* and 0.60 *w* too, and the gene pool will retain these frequencies to the *n*th generation unless disturbed.
12. In a large randomly mating population free from disturbing effects such as mutation, selection, and immigration, allele frequencies remain constant from generation to generation. Incidentally, mating does not have to be random for the preceding to be true; but we have not proved this, and in the case studied we have assumed random mating. There is also a stability of the genotypic frequencies from generation to generation under the above conditions. The genotypic frequencies are obtained from the allele frequencies, $\mathbf{p}^2WW$, $2\mathbf{pq}Ww$, and $\mathbf{q}^2ww$, if the allele frequencies are $(\mathbf{p}W+\mathbf{q}w)$. This last relationship between allele frequencies and genotypic frequencies is true only with random mating. It is known as the Hardy-Weinberg Principle.
13. The original mixed forest became subdivided into one wooded area predominantly composed of birches and another composed predominantly of pines.
14. The moth population, too, became separated into a population predominantly speckled white in color (living in the birch woods) and a separate population predominantly black in color (living in the pine woods).
15. Feeding larvae of speckled white moths on pine foliage and feeding larvae of black moths on birch foliage has no effect on the adult color.
16. There is no evidence of a decline in reproductive capacity on the part of either kind of moth in either type of woods. Discussion should bring out the possibility, proved in other insect experiments, that one of the selective factors is the presence of birds that feed on the moths; presumably the speckled white moths are less conspicuous when resting on birch bark than are black moths, and conversely, black moths are less conspicuous when resting on pine bark than are speckled white moths.
17. In the pine woods the frequency of the allele for speckled white (*w*) has diminished.

18. In the birch woods, the frequency of the same *w* allele has increased.
19. Populations that survive in a changing environment must either possess adaptive hereditary variations already, as in the case of these moths, or must be able by mutation and especially by recombination, to generate new well-adapted types rather quickly.
20. The color of the moth matches the color of the bark of the tree on which it rests.
21. Insect-eating birds.
22. Keen eyesight.
23. Place equal numbers of black and speckled white moths both in the birch forest and in the pine forest and observe the respective numbers eaten of each kind in each forest by insect-eating birds.

SUGGESTED READINGS

Books

Dowdeswell, W. H. 1960. *The Mechanism of Evolution.* Harper Torch Books, New York

Articles

Glass, B. 1953. "The Genetics of the Dunkers." *Sci. Am.* **189** [2] 76

Hardy, G. H. 1908. "Mendelian Proportions in a Mixed Population." *Science* 28:49–50

Kettlewell, H. B. D. 1959. "Darwin's Missing Evidence." *Sci. Am.* **200** [3] 48

Ryan, F. 1953. "Evolution Observed." *Sci. Am.* **189** [4] 78

Weinberg, W. 1908. "Über den Nachweis der Vererbung beim Menchen." Jahreshefte Verein f. vaterl. Naturk. in Württemberg 64:368–82

An important section of this paper is in English in C. Stern. 1943. "The Hardy-Weinberg Law." *Science* 97:137–38

FILMS

Life, Time and Change, Part X: *Natural Selection and Adaptation,* 1963, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

Life, Time and Change, Part X: *Mimicry and Other Evidence,* 1963, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

SICKLE CELLS AND SELECTION

EXERCISE
34-2

TIME: Homework exercise.

MATERIALS: None

This exercise is designed to accompany the section on selection in Chapter 34 of the textbook.

ANSWERS TO QUESTIONS

1. The chances of mating of a black male with a female of either color will be equal because there are equal numbers of the two kinds of females.
2. The same answer as for Question 1 above.
3. *WW* ♀ × *WW* ♂; *WW* ♀ × *ww* ♂;
   ww ♀ × *WW* ♂; *ww* ♀ × *ww* ♂.
4. No, each cross produces only one kind of offspring.
5. The proportions of black and speckled white moths in the offspring population will be 75% black and 25% speckled white.
6. No, it is obviously different from that which existed at the start.
7. 8. and 9. The percentages of *W* and *w* remain 50% for each.
10. The frequencies of *W* and *w* remain the same (0.5), in both the parent and offspring populations.
11. When one starts with 0.40 *WW* and 0.60 *ww* moths, the gametes are in the frequency of 0.40 *W* and 0.60 *w* too, and the gene pool will retain these frequencies to the *n*th generation unless disturbed.
12. In a large randomly mating population free from disturbing effects such as mutation, selection, and immigration, allele frequencies remain constant from generation to generation. Incidentally, mating does not have to be random for the preceding to be true; but we have not proved this, and in the case studied we have assumed random mating. There is also a stability of the genotypic frequencies from generation to generation under the above conditions. The genotypic frequencies are obtained from the allele frequencies, $\mathbf{p}^2WW$, $2\mathbf{pq}Ww$, and $\mathbf{q}^2ww$, if the allele frequencies are $(\mathbf{p}W+\mathbf{q}w)$. This last relationship between allele frequencies and genotypic frequencies is true only with random mating. It is known as the Hardy-Weinberg Principle.
13. The original mixed forest became subdivided into one wooded area predominantly composed of birches and another composed predominantly of pines.
14. The moth population, too, became separated into a population predominantly speckled white in color (living in the birch woods) and a separate population predominantly black in color (living in the pine woods).
15. Feeding larvae of speckled white moths on pine foliage and feeding larvae of black moths on birch foliage has no effect on the adult color.
16. There is no evidence of a decline in reproductive capacity on the part of either kind of moth in either type of woods. Discussion should bring out the possibility, proved in other insect experiments, that one of the selective factors is the presence of birds that feed on the moths; presumably the speckled white moths are less conspicuous when resting on birch bark than are black moths, and conversely, black moths are less conspicuous when resting on pine bark than are speckled white moths.
17. In the pine woods the frequency of the allele for speckled white (*w*) has diminished.

18. In the birch woods, the frequency of the same w allele has increased.
19. Populations that survive in a changing environment must either possess adaptive hereditary variations already, as in the case of these moths, or must be able by mutation and especially by recombination, to generate new well-adapted types rather quickly.
20. The color of the moth matches the color of the bark of the tree on which it rests.
21. Insect-eating birds.
22. Keen eyesight.
23. Place equal numbers of black and speckled white moths both in the birch forest and in the pine forest and observe the respective numbers eaten of each kind in each forest by insect-eating birds.

SUGGESTED READINGS

Books

Dowdeswell, W. H. 1960. *The Mechanism of Evolution.* Harper Torch Books, New York

Articles

Glass, B. 1953. "The Genetics of the Dunkers." *Sci. Am.* **189** [2] 76
Hardy, G. H. 1908. "Mendelian Proportions in a Mixed Population." *Science* 28:49–50
Kettlewell, H. B. D. 1959. "Darwin's Missing Evidence." *Sci. Am.* **200** [3] 48
Ryan, F. 1953. "Evolution Observed." *Sci. Am.* **189** [4] 78
Weinberg, W. 1908. "Über den Nachweis der Vererbung beim Menchen." Jahreshefte Verein f. vaterl. Naturk. in Württemberg 64:368–82
An important section of this paper is in English in C. Stern. 1943. "The Hardy-Weinberg Law." *Science* 97:137–38

FILMS

Life, Time and Change, Part X: *Natural Selection and Adaptation,* 1963, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

Life, Time and Change, Part X: *Mimicry and Other Evidence,* 1963, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

SICKLE CELLS AND SELECTION

EXERCISE 34-2

TIME: Homework exercise.

MATERIALS: None

This exercise is designed to accompany the section on selection in Chapter 34 of the textbook.

■ **The purpose of this exercise is to determine the population aspects of sickle-cell anemia and to discover how it is inherited.**

SPECIAL INSTRUCTIONS

This exercise is intended to illustrate the operation of natural selection in a human population. Since the exercise involves some student "discoveries," we suggest that it precede the discussion or textbook reading assignment of this topic.

Specifically, the exercise intends to show:

1. a clear example of the selective advantage of the heterozygote,
2. a clear example of inherited disease resistance due to a single gene, and
3. that a mutation harmful under certain conditions may be beneficial under others.

Each of these points can be elaborated in a class discussion.

There are still unsolved problems concerning the sickling gene. For example, it is not always clear why the gene is present in certain populations and absent in neighboring ones.

TECHNIQUES

Adequate illustrative bulletin board materials for this exercise can be obtained from the *Scientific American* articles cited in Suggested Readings.

This exercise can be used as homework, but should be accompanied by a classroom discussion.

ANSWERS TO QUESTIONS

1. Yes.
2. Three.
3. Three.
4. SS.
5. $S'S'$.
6. SS'.
7. SS'.
8. 25%.
9. 50%.
10. 25%.
11. $1/4 \times 1/6 = 1/24$, or approximately 4%.
12. Fewer.
13. Reduce.
14. The mutation rate of the normal allele to the sickle allele might be high enough to replace the sickle-cell alleles lost through selection.
15. For unexplained physiological reasons, heterozygotes might have more children than homozygotes.

16. Heterozygotes might be more resistant to disease than the normal homozygotes, so that more of them reach maturity, and hence leave more descendants.
17. It weakens it.
18. Weakened.
19. Symptoms: recurring fever, malaise; transmitted by bite of the *Anopheles* mosquito; symptoms produced by destruction of red blood cells by the malarial protozoan.
20. Yes.
21. Yes.
22. Yes.
23. Yes.
24. No, it may be harmful under certain circumstances and beneficial under others.
25. Yes.
26. Yes.
27. No.
28. Decrease.
29. The selective advantage of the heterozygote over both homozygotes depends upon the presence of malaria. With the disappearance of malaria, one homozygote *SS* no longer is at a disadvantage when compared with the heterozygote *SS'*. Since the other homozygote *S'S'* is still at a distinct disadvantage, the *S'* allele will be selected against and will decrease in number.
30. The answer depends upon what the student gave as an answer to Question 28.
31. No.

SUGGESTED READINGS

Books

Dunn, L. C. 1959. *Heredity and Evolution in Human Populations*. Harvard Univ. Press, Cambridge, Mass.

Articles

Allison, A. C. 1956. "Sickle Cells and Evolution." *Sci. Am.* **195** [2] 87

Gray, G. W. 1951. "Sickle-cell Anemia." *Sci. Am.* **185** [2] 56

FILMS

Genetics, Part VI: *Inheritance in Man*, No. 4, 1962, (sd, c, 28 min), p or r, AIBS (McGraw-Hill)

Genetics, Part VI: *The Treasury of Human Genes*, No. 12, (sd, c, 28 min), p or r, AIBS(McGraw-Hill)

MAMMALIAN RADIATION AND EVOLUTION

EXERCISE 36-1

TIME: Homework exercise.

MATERIALS: None

This exercise is designed to accompany the section on adaptive radiation and divergent and convergent evolution in Chapter 36 of the textbook.

■ **The purpose of this exercise is to study the principle of adaptive radiation and convergent and divergent evolution as exemplified by the orders of mammals.**

SPECIAL INSTRUCTIONS

While every attempt has been made to keep terminology at a minimum in this exercise, nonetheless the study of adaptation requires the use of terms which you may have to define for your students.

This exercise includes a check list of adaptations of mammals to five different environments and ways of life: the aquatic; the digging; the walking, running, or jumping; the arboreal; and the aerial. It is, therefore, important that several animals from each category be selected in order to show adaptive radiation and divergent and convergent evolution within the group. Representative animals of each group are listed in the table, page 245, Representative Mammals from Five Different Environments.

When two originally similar organisms (carnivores) evolve to become less similar (cat and seal) the phenomenon is one of divergence. Divergence is very common and, indeed, is the necessary basis for the whole diversity of the living world. There can be no convergence unless there has been previous divergence.

The most conspicuous examples of convergence occur wherever two distantly related organisms come to resemble one another. The similar body forms of a fish and a porpoise are good examples of convergent evolution. Convergence is accounted for by the selective action of a common or a simple environment on different animals. They gradually become adapted to it in the same way.

Adaptive radiation is exhibited by a group of organisms which spreads out into the environment and becomes well adapted in many different ways to exploit certain habitats and ecological niches. The adaptive radiation of the Australian Metatherian mammals into almost every conceivable environmental niche is a classic example. Another example is the adaptive radiation of the Galapagos finches discussed in the text.

TECHNIQUES

This exercise gives an excellent opportunity for a trip to a zoo or a museum, whether as an individual assignment or by a class field trip. If a trip is not possible, pictures could very well be used for this exercise. Pictures from popular magazines, such as "Natural History," glued to cardboard to prevent tearing, and then either placed on a bulletin board or passed from student to student for observation, may serve in lieu of a field trip.

While this exercise can be done outside of class, nonetheless, there should be a classroom discussion of the results.

The form for reporting the data at the end of this exercise may be duplicated and distributed to the students or placed on the board for them to copy in their notebooks.

You may wish to introduce the phenomenon of parallel evolution which relates to structures which evolve independently but along similar lines in related forms, such as the incisor teeth of Lagomorphs and those of Rodents. Here again the selective action of a similar environment on a common genetic potential is the basis for the phenomenon.

SUGGESTED READINGS

Books

Colbert, E. H. 1955. *Evolution of the Vertebrates.* Wiley, New York
Dobzhansky, T. 1955. *Evolution, Genetics and Man.* Wiley, New York
McSpadden, J. W. 1947. *Mammals of the World.* Garden City, New York
Sanderson, I. T. *Living Mammals of the World in Color.* Garden City, New York
Wallace, B. and A. M. Srb. 1961. *Adaptation.* Prentice-Hall, Englewood Cliffs, N.J.
Young, J. Z. 1957. *The Life of Mammals.* Oxford Univ. Press, London

Articles

Simpson, G. G. 1945. "The Principles of Classification and a Classification of Mammals." *Bull. Amer. Mus. Nat. Hist.* No. 85. New York

FILMS

Animals Unlimited, 1950, (sd, b&w or c, 20 min), FON
African Fauna, 1957, (sd, c, 11 min), Walt Disney
Arctic Borderlands in Winter, 1948, (sd, b&w, 11 min), p, Coronet
Beaver Valley, 1953, (sd, c, 32 min), p or r, Disney
Seal Island, 1953, (sd, c, 27 min), p or r, Disney

MAMMALIAN ADAPTATION

Groups of Mammals Adaptations	*Metatheria (Pouched Mammals)*	**EUTHERIA** Orders of Placental Mammals				
		Primates	*Rodentia*	*Carnivora*	*Artiodactyla* *Perissodactyla*	*Cetacea*
AQUATIC (swimming)						
1. Streamlined body						
2. Web-footed; or flippers						
3. Flattened tail						
4. Sense organs placed high on head						
5. Loss or reduction of hind limbs						
FOSSORIAL (digging)						
1. Forefeet for digging						
2. Ears and eyes reduced in size						
3. Short legs						
4. Prehensile snout and tusks						
CURSORIAL (moving on land)						
1. Large eyes and ears						
2. Running on toes						
3. Tail for balancing						
4. Limbs for jumping						
ARBOREAL (tree climbing)						
1. Prehensile tail						
2. Tail for balancing						
3. Claws, sometimes retractile						
4. Opposable thumb						
5. Toes separated for grasping						
AERIAL (flying)						
1. Skin modified for gliding						
2. Active flying						

REPRESENTATIVE MAMMALS FROM FIVE DIFFERENT ENVIRONMENTS

			EUTHERIA (Placental Mammals)			
Environment	*Metatheria (Pouched Mammals)*	*Primates*	*Rodentia*	*Carnivora*	*Artiodactyla Perissodactyla*	*Cetacea*
AQUATIC (swimming)	water opossum		beaver capybara muskrat	otter seal walrus sea lion		whale porpoise dolphin
FOSSORIAL (digging)	wombat marsupial mole		pocket gopher ground squirrel prairie dog lemming marmot	badger		
CURSORIAL (running or jumping)	bandicoot Tasmanian wolf kangaroo	baboon	kangaroo rat chipmunk jerboa agouti	wolf fox coyote lion dog	antelope deer giraffe bison elk horse	
ARBOREAL (tree-living)	koala phalanger opossum	lemur monkey	porcupine gray squirrel flying squirrel	lynx jaguar wildcat		
AERIAL (flying)	The bats, Eutherian order Chiroptera, are the only true flying mammals. Others, such as the flying squirrel (Rodentia) and flying phalanger (Metatheria) glide from place to place but do not really fly.					

THE STUDY OF FOSSIL PLANTS

EXERCISE 36-2

TIME: One laboratory period.

MATERIALS: See page 263 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on fossils in Chapter 36 of the textbook.

■ **The purpose of this exercise is to give the student an opportunity to work with fossils and thus to become aware of the structural details preserved in some fossils and to learn the techniques that can be used in preparing fossils for study.**

SPECIAL INSTRUCTIONS

This has been a highly successful exercise exciting great student interest and enthusiasm. The fact that biologists can tell about the internal as well as the external forms of extinct organisms adds a new dimension to the examination of fossils in the eyes of the students. The making of the fossil peel can be dramatized by pointing out to the student that he is going to see something that no one else has seen before.

A compression fossil is the carbonized remains of the organism formed by the weight of the surrounding matrix. Usually there are visible remains of the organism in the form of a carbon film on the fossil surface.

An impression fossil is the imprint that the compression fossil has made in its surrounding rock matrix.

TECHNIQUES

This exercise calls for working with two different concentrations of hydrochloric acid which must be clearly marked and kept separate so that they are not confused. The student should be cautioned about the corrosive properties of acids and instructed not to get them on skin or clothing.

The acetate film can be purchased in clear sheets 0.002 x 20 x 50 inches from: Colonial Kolonite Co., 2232 W. Armitage Ave., Chicago 47, Ill. Carborundum silicon carbide grit No. 400 can be purchased from the Carborundum Co., 3335 W. 47th St., Chicago, Ill.

Coal balls can be obtained from biological supply houses or from Dr. Wilson Stewart, Department of Botany, University of Illinois, Urbana, Ill.

ANSWERS TO QUESTIONS

1. The fact that there are common patterns in organisms helps in reconstructions. Homologous parts and patterns serve to group fossils just

as they do living forms. The similarities of many fossils to living organisms also provide valuable clues to organization. Once the unknown fossil parts are related to some known fossil or living organism, the use of homologous structural patterns serves as the best clue to reconstructions.

2. It would decay, and fossilization would not be possible.
3. The acid effervesces on the shell, which is probably either calcium or magnesium carbonate. There is no reaction on the plant in the shale or on any fossil that is primarily silica.
4. They may be marine in origin, as are the carbonates, or terrestrial in origin, as are the sandstones and shales. The sediments also differ in the size of the particles of which they are composed.
5. The acid attacks the surrounding carbonate, but not the organic plant material imbedded in it.
6. The vascular tissue, especially the cell walls of the xylem, leaves with heavy cuticle, and other thick-walled tissues.
7. We can learn the cell and tissue structure of ancient plants – both their internal organization as well as their external appearance.

SUGGESTED READINGS

Books

Andrews, H. N. 1957. *Ancient Plants and the World They Lived In.* Comstock, Ithaca, N.Y.

Arnold, C. A. 1947. *An Introduction to Paleobotany.* McGraw-Hill, New York

Beerbower, J. R. 1960. *Search for the Past: An Introduction to Paleontology.* Prentice-Hall, Englewood Cliffs, N.J.

Colbert, E. H. 1961. *Dinosaurs, Their Discovery and Their World.* Dutton, New York

Dunbar, C. O. 1949. *Historical Geology.* Wiley, New York

Harris, T. M. 1957. *How We Study Fossil Plants.* New Biology, Vol. 22, Penguin Books, Baltimore, Md.

Moore, R. C. 1949. *An Introduction to Historical Geology.* McGraw-Hill, New York

Thomas, G. 1950. *Processes of Fossilization.* New Biology, Vol. 8, Penguin Books, Baltimore, Md.

Articles

Abelson, P. H. 1956. "Paleobiochemistry." *Sci. Am.* **195** [1] 83

Deevey, E. S., Jr. 1952. "Radiocarbon Dating." *Sci. Am.* **186** [2] 24

Janssen, R. E. 1948. "The Beginnings of Coals." *Sci. Am.* **179** [1] 46

Taylor, T. N. 1962. "The Coal Ball Peel Technique." *Fast Journal.* April-May.

FILMS

Hunting Animals of the Past, 1951, (sd, c, 22 min), Nebraska

Journey into Time, 1960, (sd, b&w or c, 14 min), p, Sterling

Prehistoric Times; *The World before Man*, 1953, (sd, b&w or c, 11 min), p, Coronet

Life, Time and Change, Part X: *Dinosaurs*, No. 10, 1963, (sd, c, 29 min), p or r, AIBS(McGraw-Hill)

Ecology

The following three exercises are specifically designed as field work. It seems fitting and proper that the student should have the opportunity in either late spring or early summer to see organisms in their natural environments and to understand the organization of organisms into populations and communities.

These exercises can be a fitting culmination to the year of biology laboratory experiences if properly planned. It is essential that you, as a teacher, read and understand the exercises thoroughly in advance, and find suitable areas in a relatively immediate vicinity.

These exercises give not only an opportunity for observation, but also for collecting some specimens that can be used in future years as a collection of typical plants and animals from your area.

The following simple rules are suggested for all field work. Observing them will make your task simpler than it might otherwise be.

1. Be sure you know your federal and local game laws before collecting fish, birds, or mammals.
2. Always seek permission before invading private property.
3. Avoid collecting or disturbing eggs, young, or nests of birds and other animals.
4. When a log has been rolled over to search for specimens, roll it back to its original position when you are through looking under it. Replace rocks, wood, or any litter that has been disturbed. These serve as a blanket and moisture-holder for many kinds of living things. Save lives no matter how small.
5. Always keep to a minimum the destruction of tree branches or entire plants, and do not litter or deface the area in which you are working.

A PLANT COMMUNITY

EXERCISE
39-1

TIME: Up to 3 periods for field work, 1 to 2 laboratory periods for analysis and calculation, plus up to 3 periods to prepare summary records and for class discussion.

MATERIALS: See page 267 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on communities, in Chapter 39 of the textbook.

■ **The purpose of this exercise is to study the interrelationships of plants in the nearby field or forest. Students will study the diversity, abundance, and density of the organisms in the selected area and will attempt to evaluate their functional relationships in the community.**

SPECIAL INSTRUCTIONS

This exercise is designed as an illustrative study of a terrestrial community. It is intended to point up the community as a unit composed of populations which affect one another. It should illustrate the pyramid of numbers and the great diversity of living forms. The summary should point out the stratification of the organization of the biome.

The exercise will, in many cases, prove to be too long to be done in its entirety. The length of the exercise results from an attempt to anticipate a great variety of environmental conditions throughout the United States. The teacher, therefore, if time is limited, should choose those parts of the exercise commensurate with the principles to be demonstrated, which can be effectively undertaken in the immediate area in which he is located.

This exercise has been found to work very well with either of the following two plans of action. Teams of 8 or 16 (most successful for entire laboratory group) were set up and captains appointed. The captains were taken to the selected spots, preceding the exercise, and briefed on what they were to do. Then the entire teams, after being briefed by the captains, went out during class period and carried out the field work.

You may find it more satisfactory to appoint selected students to a Field Team and on a Saturday have them collect for the entire class. However, the exercise is designed for a half-day in the field. With adequate preparation minimum collections can be made in one period from a field relatively close to the school.

The amount of time required will depend upon the degree of thoroughness desired and upon the nature of the plot being studied. Do not underestimate the time required, however. The estimated time, based on a 55-minute period, is:

FIELD:

1. Field trip to choose study site and lay out pattern for study area. One period.
2. Measurement of vegetation in quadrats and collection of plants. One to two periods.

LABORATORY:

3. Analysis of plant abundance and distribution of field data. Calculations. One to two periods.
4. Discussion and completion of summary reports. One period.

DISCUSSION:

5. Correlation and emphasis of principles. One to two periods.

It is essential that you understand the selected area thoroughly, in terms of what is to be done in this exercise, before the students are assigned to this field problem. You should know the common plants of the region, and be prepared to answer questions which will arise.

Before you take the class into the field, the students should be thoroughly briefed on the procedures to be used by each team in order to work in an efficient manner and to understand what they are doing.

The field trip may be taken after school hours, but it is better to schedule it on a Saturday morning or afternoon. Some teachers have attempted to take the class on an all-day field trip, but it is difficult to maintain interest and productive field work for so extended a period.

Certain parts of the laboratory exercise call for the work to be done by pairs of students. The instructor should assign the student pairs. The instructor must also select and assign the species of organisms to be studied by the students, some particular shrubs and herbs, for example. The laboratory directions also call for students to collect and preserve certain plants. The species to be collected must be assigned by the instructor and collection and preservation techniques explained by him.

TECHNIQUES

As we previously stated, the area should be thoroughly familiar to you before making the field trip. It is advantageous to have prepared in advance a duplicated list of common plants of the area that you expect the students to observe, together with field instructions. If possible, prior to the field work, you should collect representative plants from some nearby area with the same flora. This collection, displayed in class prior to the field trip, will acquaint the students with representative species they will see and will make the exercise more meaningful.

You should standardize the form of the records you wish students to keep so that they will be comparable. After the field study is complete, the records of each team of students should be duplicated and given to the entire class for class analysis as indicated in the student guide.

The following flow sheet, Figure 39-1-1 suggests a method of breaking down the field collections.

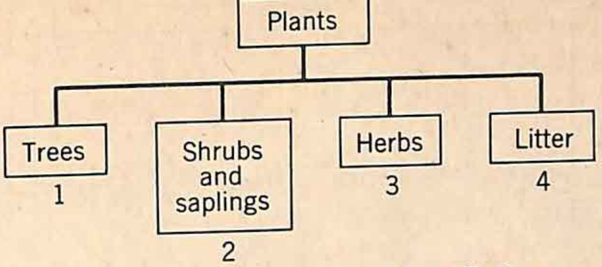

Figure 39-1-1 **Flow chart for plant collection.**

We have attempted to give enough choice of methods to give this exercise flexibility as well as detailed instruction. You will need to decide which method to use according to your own situation.

The Site. The area selected should be near your school so you can return easily to work on several different days. You may wish the class to select two different kinds of habitats for comparative purposes. The site you select should be as nearly as possible a climax stand representative of your local biome (desert, grassland, taiga, deciduous forest, etc.). In metropolitan areas, a simpler community such as a lawn, playground, vacant lot covered with tall grass, road shoulder, etc., may be used just as adequately.

Once you have chosen the type of area to study, you must choose the exact site to study. This can be done in one of three ways:

SUBJECTIVE CHOICE. You simply look the area over and decide subjectively that you want the position of your quadrats to be in certain places of convenience or suitability.

RANDOM LOCATION OF STUDY SITE. You can lay the entire area out in blocks and assign each block a number. Mark a series of these numbers on pieces of paper, put them in a hat, and draw a number for each team. The number will then tell the location of their study site.

SYSTEMATIC LOCATION OF STUDY SITE. You can do this by starting at a certain point and laying off a line in some direction (N, E, S, or W). Every 20 m on this line (or some convenient number of paces) you can assign a team. They then use the line you are walking as one side of their quadrat.

Either of these last two methods is preferable to the first. Perhaps you will have time to explain to the class the importance of subjective bias in sample selection.

The size of the site will depend on the density of the vegetation. The suggested 50-m size in the student guide may be used as it is a more convenient area for a class. However, in areas of dense vegetation, other quadrats may be used, as those shown in Fig. 39-1-2.

TREE QUADRAT. A square 10 x 10 m within which you count all trees that have trunks more than 10 cm in diameter at breast height (d.b.h.).

SHRUB AND SAPLING QUADRAT. The size should be 2 x 10 m and within

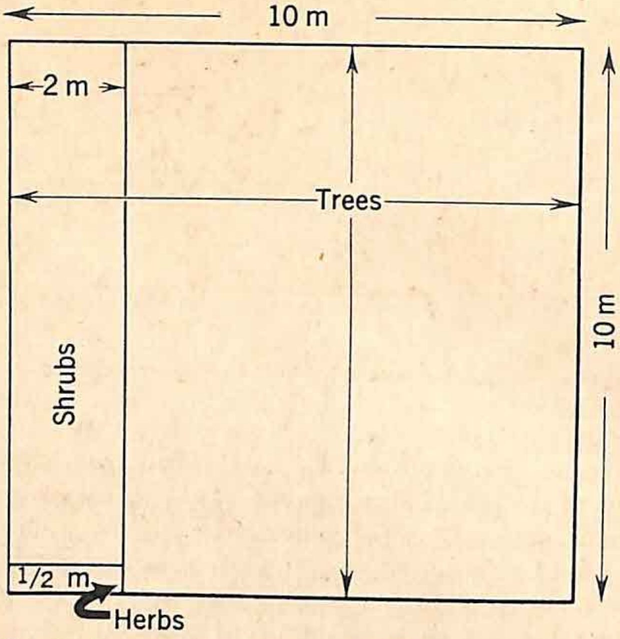

Figure 39-1-2 **A convenient-sized quadrat for areas of dense vegetation.**

this you count all shrubs (low woody plants) and all young trees less than 10 cm and more than 2.5 cm d.b.h.

HERBACEOUS PLANTS AND TREE SEEDLINGS. This quadrat should measure 0.5 x 2 m and here you count all herbs and tree seedlings. You can also estimate the amount of the ground that is (a) bare, (b) covered by fallen leaves (litter), or (c) covered by lichens and mosses.

Measurement of Vegetation

Cover is a measurement of the relative amount of ground covered by the leaves and stems of a plant's canopy. This can be estimated by measuring the amount of one of the 10-m boundary lines of your quadrat that is underneath each shrub species. For example, in Fig. 39-1-3, shrub species A has a cover value of $2 + 1 = 3$ m, species B has $0.75 + 1.75 = 2.5$ m, and species C has 2.25 m. Open space around the shrubs is about 2.5 m. The number and size of each kind of tree sapling and tree seedling also can be estimated by the small quadrats. The herb, lichen, moss, and litter cover can be estimated roughly as a percentage of the small 0.5 x 2-m plot. As an aid to estimating, you can measure the area covered by your hand in proportion to the total plot area.

The site profile is to be done by the *entire* student group. Then each subsequent part will be assigned to a particular student team.

The coverage of trees can be computed in one of two ways: (1) measurement of diameter of crown as estimated above, or (2) measurement of stem diameter which is converted to basal area. The forestry service uses

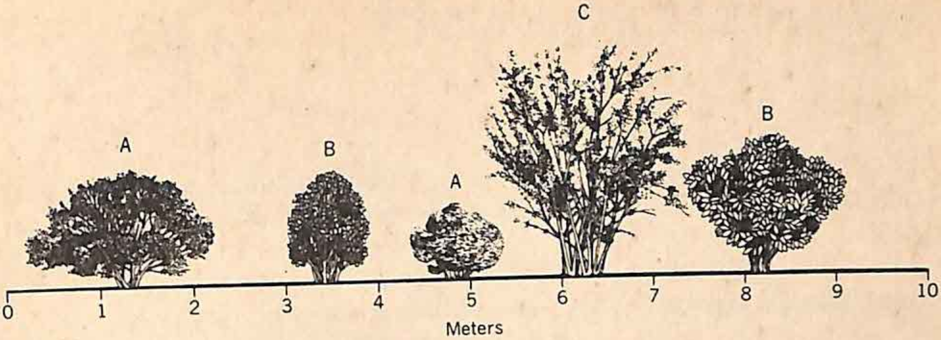

Figure 39-1-3 **Method of taking cover measurement.**

charts to convert stem diameter to crown coverage. The "tree" squads may collect leaf, twig, and bark samples for later study.

Measurement of herb and shrub cover by the crown-diameter method requires the least expenditure of time, since all the measurements can be made in the field. Computation of the cover can be made at home and reported to the class.

Plants can be preserved by pressing them between layers of newspaper in a plant press or under a heavy weight. Several changes of newspaper may be necessary if the plants are moist.

ANSWERS TO QUESTIONS

1. through **5.** Depend upon student data.

6. The tree which forms the present climax vegetation.

SUGGESTED READINGS

Books

Daubenmire, R. F. 1959. *Plants and Environment*. Wiley, New York

Philips, E. A. 1959. *Methods of Vegetation Study*. Holt, Rinehart and Winston, New York

Polunin, N. 1960. *Introduction to Plant Geography*. McGraw-Hill, New York

Articles

Went, F. W. 1955. "The Ecology of Desert Plants." *Sci. Am.* **192** [4] 68

FILMS

The Community, 1962, (sd, b&w or c, 11 min), p or r, EBF

The Desert, 1962, (sd, b&w or c, 22 min), p or r, EBF

The Grasslands, 1962, (sd, b&w or c, 17 min), p or r, EBF

The Temperate Deciduous Forest, 1962, (sd, b&w or c, 16 min), p or r, EBF

World in a Marsh, 1958, (sd, c, 22 min), p or r, McGraw-Hill

Ecology, Part IX: *Tundra Ecology*, No. 7, (sd, c, 28 min), p or r, (AIBS)McGraw-Hill

Ecology, Part IX: *The Deciduous Forest*, No. 8, (sd, c, 28 min), p or r, (AIBS) McGraw-Hill

Ecology, Part IX: *The Tropical Rain Forest*, No. 9, (sd, c, 28 min), (AIBS)McGraw-Hill

Ecology, Part IX: *Grasslands and the Desert*, No. 10, (sd, c, 28 min), p or r, (AIBS) McGraw-Hill

THE ANIMAL COMMUNITY

EXERCISE 39-2

TIME: One half day for field work, one to two laboratory periods, plus homework time to prepare summary records and one period for class analysis.

MATERIALS: See page 271 in the *Student Laboratory Guide.*

This exercise is designed to accompany the section on communities in Chapter 39 of the textbook.

■ **The purpose of this exercise is to study the interrelationships of animals within a plant community. The students will study the distribution, diversity, abundance, and density of the animals of a selected area and will attempt to evaluate their functional relationships in the community.**

SPECIAL INSTRUCTIONS

If you have already done Exercise 39-1, special instructions applicable for studying the plant community are also germane to studying the animal community, and should be reviewed at this time.

In collecting flying insects the 3 : 1 mixture of gasoline-chloroform can be prepared beforehand and taken into the field in a dropper bottle. Make sure the students understand that this is an explosive mixture and must not be used near an open flame or sparks.

Binoculars will be necessary for the best observation and identification of birds, preferably a 6 × 30 or 7 × 35 size.

You may wish to do both Exercise 39-1 and 39-2 during the same field period. This is feasible by dividing the class into two groups, one working on Exercise 39-1, the other on Exercise 39-2. However, to accomplish both exercises during the same field period will entail a great degree of organization, preparation, and supervision.

TECHNIQUES

Review the general instructions under Techniques for Exercise 39-1. Figure 39-2-1, following, suggests a method of breaking down the animal collections called for.

Should you desire to combine Exercises 39-1 and 39-2, the following are possible student combinations for minimum collections. The significance of the numbers is apparent when Figure 39-1-1 is compared with Figure 39-2-1.

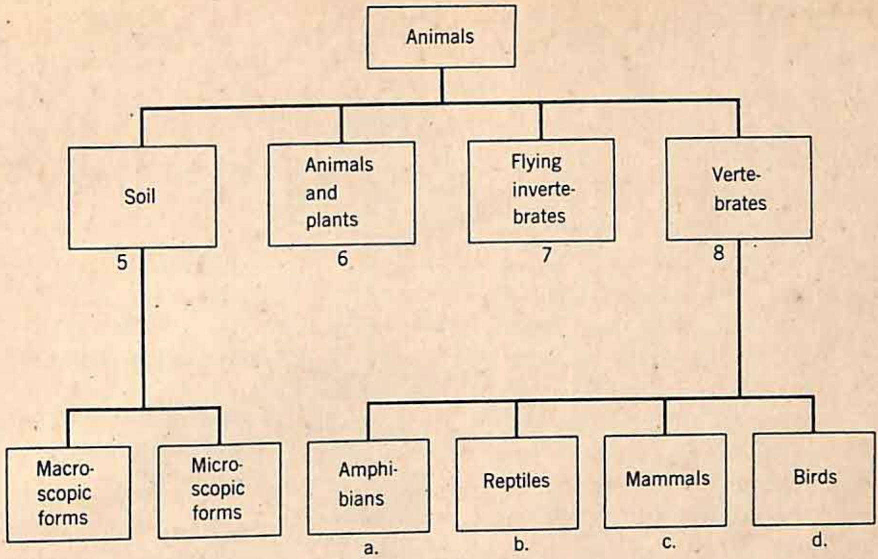

Figure 39-2-1 **Flow chart for animal collection.**

I. 1 and 8c, d.
II. 2 and (6 or 7 or 8c, d)
III. 3 and (6 or 7 or 8a, b, c)
IV. 4 and (5 or 8a, b)

Two suggested forms for field data for the study of the animal community are shown at the end of this exercise.

Remember to emphasize to the student to watch for the relationships between the animal community and the plant community.

The use of a small can for gathering soil samples will work *if* the soil is moist. If the soil is *not* moist, then use a trowel or a bulb trowel. A sharpshooter spade (a trenching tool) and a posthole digger are also suggested as substitutes.

Figure 39-2-2 shows the construction of the modified Berlese funnel to be used in collecting microscopic soil animals. While the directions in the student guide are clear, nonetheless the principle of using heat and drying to drive the small animals down through the soil and out into the container of formalin should be reviewed. The animals should be collected daily for about a week and identified under a dissecting or a compound microscope. The record of the numbers and species of animals obtained each day over a period of a week will provide interesting data which can be graphed for visual summarization.

A collecting net can be made of a stiff wire coat hanger and a piece of cheesecloth by bending the hanger into a hoop and using the hook of the hanger as a handle. The cheesecloth is made into a cone or bag which is sewed to the hoop.

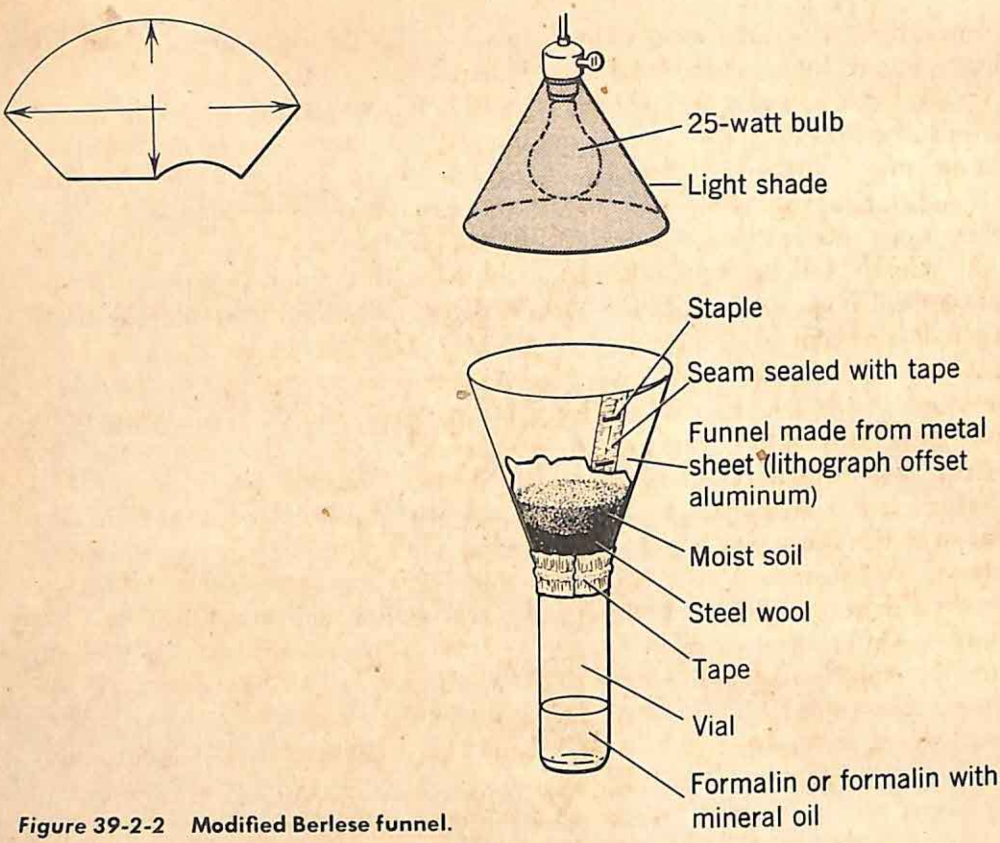
25-watt bulb
Light shade
Staple
Seam sealed with tape
Funnel made from metal sheet (lithograph offset aluminum)
Moist soil
Steel wool
Tape
Vial
Formalin or formalin with mineral oil

Figure 39-2-2 **Modified Berlese funnel.**

The standard insect killing jar contains potassium cyanide. Since the crystals and fumes are deadly, it may be more advisable to buy the jars already made. If you make your own jars, pea-sized bits of potassium cyanide should be sprinkled into the bottom of several different size bottles, and covered with dry plaster of paris and then covered with a wet layer of plaster of paris. (CAUTION: *Never let potassium cyanide touch the skin. Never breathe the fumes.*) Covering the hardened plaster with one or two thin sheets of blotting paper tends to absorb moisture.

As both the fumes and crystals of KCN are deadly poisonous, it may be advisable to use a substitute. Use a jar or wide-mouthed bottle and fasten a wad of cotton saturated with carbon tetrachloride (also poisonous and can be absorbed through the skin) or cigarette-lighter fluid to the inside lid or cork. Or a more elaborate kind of killing jar can be made by packing rubber bands into the bottoms of jars of several different sizes and soaking them with carbon tetrachloride. Cover this layer with absorbent cotton packed tightly and cover this with a circle of cardboard or thin disks of cork to hold the materials in place. Periodic replacement of the carbon tetrachloride is necessary.

Instant-coffee jars or fruit jars, or bottles with tight lids are satisfactory for the transportation and storage of preserved specimens. The most desirable preservative is 70% ethyl alcohol. In case this is not available, 5–8%

formalin is also satisfactory. *Do not use methyl or wood alcohol.* Certain large, winged insects are best transported in paper envelopes.

Vertebrates should be preserved in 10% formalin. Fish and amphibians should be killed by dropping them directly into the formalin. Other vertebrates may be killed in this manner or by injecting them with a few drops of formalin. Ether and chloroform should not be used for vertebrates because they may cause the specimen to become distorted or the color to fade. After death, large fish, toads, and all reptiles, birds, and mammals should have their body cavities filled with 10% formalin. This may best be done by inserting a needle into the lower portion of the belly and filling the body cavity with formalin from a syringe. Large animals will need several injections. Any snake over 45 cm long, for instance, should have an injection into the body cavity every 5 cm from anus to head. Animals with legs, except for frogs and salamanders, should have all four legs injected. If a syringe is not available, a razor blade may be used to make 3 cm long slits through the body wall. A slit should be made at the same points indicated above for the injections. Once an animal has been injected or slit open, it should be placed in whatever position you desire it, and allowed to harden. After the animal is injected, it can harden in the air. If it is slit open, it must be placed in a tray or jar of 10% formalin for hardening. Hardening takes from 1 to 3 hours, depending on the size of the animal. After hardening, the animal should be sealed in a container of 10% formalin or 70% ethyl alcohol or 50% isopropyl alcohol.

If you are using traps for catching small animals, never reach into a trap without looking carefully beforehand to avoid being bitten or scratched.

ANSWERS TO QUESTIONS

1. Depends upon student data.
2. The general trend is that the animals increase in size and decrease in numbers as we go from first-order consumers through *n*th-order consumers.
3. through 8. Depends on student data.
9. The first-order consumers should be in greater number.
10. Depends on student data.

SUGGESTED READINGS

Books

Bates, M. 1962. *Animal Worlds.* Random House, New York
Buchsbaum, R., and M. Buchsbaum. 1957. *Basic Ecology.* Boxwood Press, Pittsburgh
Dowdeswell, W. H. 1961. *Animal Ecology.* Harper Torch Books, New York
Morholt, E. *et al.* 1958. *A Sourcebook for the Biological Sciences.* Harcourt, Brace & World, New York
Chapter 15 covers field work and collecting.

FILMS

Chain of Life, 1953, (sd, c, 11 min), p or r, Pictura
Nature's Half Acre, 1953, (sd, c, 33 min), p or r, Disney
Vacant Lot, (sd, c, 21 min), p or r, IFB

SAMPLE DATA SHEET FOR STUDY OF AN INVERTEBRATE ANIMAL COMMUNITY

		Type of Sample				
		Soil Extraction				
Subplot	Kind of Animal	Sub-surface	Litter	Macroscopic Forms	Animals on Plants	Flying Insects

SAMPLE DATA SHEET FOR STUDY OF A VERTERBRATE ANIMAL COMMUNITY

Subplot	Kind of Animal	Number in Subplot	Type of Plant Community in which Found	Layer of Plant Community in which Found

ECOLOGICAL SUCCESSION

EXERCISE

39-3

TIME: One or two laboratory periods.

MATERIALS: See page 275 in the *Student Laboratory Guide*.

This exercise is designed to accompany the material on ecological systems in Chapter 39 and on ecological succession in Chapter 8 of the textbook.

■ **The purpose of this exercise is to observe different stages in plant and animal succession in two contrasting plant communities, one in a very wet habitat, and the other in a dry habitat. The student will also be given an opportunity to observe the stages in a series of changes that take place in the establishment of a plant community characteristic of a particular climate.**

SPECIAL INSTRUCTIONS

This exercise is designed to demonstrate ecological succession over a long period of time. By careful selection of study areas it should be possible for students to observe a number of communities in the succession. It should be remembered, however, that each community is a passing phase that will, in time, be replaced by another community.

Select an area, if possible, that has a pond with an adjacent hillside or field containing large stones. The pond may be man-made or natural, but it should be a permanent pond that has been undisturbed for three or four years. No digging should be done without permission. An old cemetery, rock outcrop, or rocky field will be suitable for Part B. A public cemetery, such as a war memorial, may be used. If you select a private or organization-controlled cemetery for the study, permission should be obtained first.

One period may be given to the hydrarch (wet) succession study and one to the study of xerarch (dry) succession, or both may be combined in a single period if suitable sites are adjacent.

Each student should have a field notebook, a 20-cm ruler, and a pencil.

Pond (Hydrarch) Succession

The older the pond, the more stages in succession are likely to be present. Even if a pond is young (3-10 years), however, the first few stages in the succession should be present.

We can presume that the pond started with barren water in a barren basin.

There are animals characteristic of each of the communities in and around the pond just as there were certain animals characteristic of the various communities in the previous exercise. Call attention to a few easily recognized animals of each community.

If the study is made in a man-made pond, try to find the age and original dimensions of the pond. The students should then measure the width of the various communities and the depth and diameter of the entire pond. With all of this information, the students may be able to calculate the date at which the pond will be completely filled in if succession is permitted to run its course. They may also use these data to estimate the ages of the various communities.

Dry (Xerarch) Succession

Students may have difficulty locating the various stages because they are not as conveniently arranged as in pond succession. Emphasize to them that the condition of the stone's surface determines what community will invade first. A smooth surface like a polished tombstone could be invaded only by crustose lichens. On the other hand, a stone with a rough surface may be initially invaded by higher forms of life.

Animals are associated with these communities, but they may be quite inconspicuous.

If this type of succession is studied in a cemetery, the dates on the headstones may be used to obtain some idea of the life span of the various communities. To do this with a minimum amount of error, work should be limited to an old section of the cemetery and to unpolished native stones. The stones should be either all shaded or all unshaded. That is, the stones used should all be in comparable areas.

In general, large natural areas of rock outcrops provide the best sites for studies of xerarch succession. If these are not available, abbreviated studies can be carried out in the sites suggested above.

Be sure the students understand the exercise and are adequately briefed on what they are to do in the field before they are taken to the site.

It is essential that you visit the selected areas beforehand and become familiar with the animal and plant communities which are present.

Representative collections of animals and plants made prior to the field exercise, and displayed in the laboratory, will expedite the student's work in the field.

Duplicated lists of common plants and animals of the area to be studied, together with outline maps of the region on which students can add the vegetation or animal life they observe will prove helpful.

ANSWERS TO QUESTIONS

1. and 2. Depend on student observations.
3. The vegetative parts of these plants tend to be single filaments or finally branched filaments.
4. They are shaped to take maximum advantage of the light which filters down to them through the water and do not shade one another as broad leaves might well do.
5. Depends on student observations.
6. In the more shallow water, where the growth of vegetation slows wave and current action and allows sediments to deposit at a faster rate.

7. It becomes increasingly smaller in diameter as the vegetation moves inward year by year and shallower and shallower with the deposition of sediments.
8. Depends on student observations.
9. The submerged aquatics beneath the floating leaves are not so abundant since the floating leaves shade the area beneath them and make it a less desirable area for photosynthesis.
10. The floating aquatic plants provide a cover under which animals can hide in relatively shallow water.
11. They move toward the center of the pond because of the deposition of sediments around the edges.
12. The climatic climax is determined by the climate of the area and the soil conditions.
13. A fire which would burn away the vegetation on the banks of a pond would increase the water runoff into it, and consequently increase the rate of sedimentation; an increase in rainfall would increase the rate of sedimentation; a drier environment would decrease the rate; a heavy cover of vegetation in the uplands around the pond would decrease the rate of sedimentation; a rapid dying of vegetation in the pond would increase the depth of sediment; etc.
14. This will depend upon student observation, but usually the area becomes too dry for the maintenance of the preceding vegetation, and the aquatics are forced to follow the receding water line. The temperature on land will be above that in the water.
15. Because it is in equilibrium with the physical environment.
16. If the environmental conditions, such as temperature, rainfall, or soil should change.
17. The crustose lichen community will have the least amount of soil, the foliose and fruticose lichens will have a greater amount of soil. The amount must be determined by the student.
18. The crustose lichens have begun to accumulate soil required by the larger leaflike lichens, which then grow more rapidly and crowd out the less well adapted crustose lichens.
19. These plants are capable of drying out and still remaining viable. When water returns, the plant commences to grow again.
20. The plant body requires relatively smaller amounts of moisture, minerals, and sunlight if it can complete its life cycle within a few weeks. Therefore, small plants are successful in areas with severe climatic extremes, short growing seasons, poor soils, or limited amounts of water.
21. The annual herbaceous plant community requires more soil than lichens or mosses, but less than perennials, shrubs, and trees.
22. (a) Some of them develop succulent parts in which water can be stored. (b) Some have few leaves from which water evaporates. (c) Some may shed their leaves or dry up during the hot summer. (d) Some may grow along the only available sources of water present in a normally dry habitat.

23. Will depend on student observations, but probably there will be some.

24. The shrub community is one of greater depth of soil, and the shrubs, in part, will shade herbaceous annuals or perennials, which will reduce evaporation. The shade and smaller water loss will encourage the growth of herbs requiring more moisture and more tolerance of shade.

25. Depends on student observations.

26. The young plants will be tolerant of shade and the community as a whole will persist over the area for an extensive length of time.

27. They require more moisture, are tolerant of shade, have a less highly developed cuticle, and are larger and leafier; especially having bigger and broader leaves.

28. Depends on student observations, but probably yes.

29. The forest offers cover for protection and it offers food.

30. The soil is the more important, for the climate (except for the microclimate) will not change for long periods of time. For example, the temperature and humidity may be the same on a barren rock as at the surface of the ground in a deciduous forest. The soil in this case is the determining factor, not only because of its nutrients but because of its moisture content.

SUGGESTED READINGS

Books

Clements, F. E. 1963. *Plant Succession and Indicators.* Hafner, New York
A reprint of a 1928 classic.

Daubenmire, R. F. 1959. *Plants and Environment.* Wiley, New York

Oosting, H. J. 1948. *The Study of Plant Communities.* W. H. Freeman, San Francisco

Articles

Deevey, E. S. 1951. "Life in the Depths of a Pond." *Sci. Am.* **185** [4] 68

Platt, R. 1956. "Flowers in the Arctic." *Sci. Am.* **194** [2] 88

Went, F. W. 1955. "Ecology of Desert Plants." *Sci. Am.* **192** [4] 68

FILMS

The Pond, 1961, (sd, c, 20 min), p or r, IFB
The Spruce Bog, 1958, (sd, c, 23 min), p or r, McGraw-Hill
Seeds of Destruction, (sd, b&w, or c, 10 min), p or r, EBF
Beaver Valley, 1953, (sd, c, 32 min), p or r, Disney
Succession: *From Sand Dune to Forest,* 1961, (sd, b&w or c, 16 min), p or r, EBF

DIRECTORY OF FILM PRODUCERS AND DISTRIBUTORS

The following abbreviations are used in the film listings at the end of each exercise. In addition, each film listing includes, wherever available, date of release, supplier, and other pertinent information.

b&w – black and white
c – color
fs – filmstrip
min – minutes
p – purchase
r – rent
sd – sound
si – silent

The following list of film producers and major distributors (along with their abbreviations) are those cited in this publication at the end of each exercise. We do not believe that films should be ordered for use in the classroom from catalogue descriptions. Instead films should be previewed by the teacher. In many instances the entire film may be applicable while in other instances only a small segment will be useful for a particular exercise or for a point to be illustrated.

Each film should be judged alongside each particular class and its needs, as well as the needs of the exercise. It should be used as an integral part of the exercise, not as something to fill in time.

Many more films have been cited than can possibly be used. Some films are destined to become devices for lucid explanations of particularly difficult points. These so destined will be determined by the individual teacher in relation to his own classes.

If your school system has an audio-visual center, many of these films may be immediately available. Or you may wish to indicate to your center those films that should be a part of a good biology film library because of their continued valuable use. The BSCS *Biology Teachers' Handbook* published by John Wiley & Sons, New York City, discusses films in greater detail than is possible here.

AIBS McGraw-Hill
    Text Film Division, McGraw-Hill Book Co., Inc., 330 W. 42nd St., New York 36, N.Y.

Almanac
    Almanac Films, Inc., 516 5th Ave., New York 18, N.Y.

Bajer
    Dr. A. Bajer, Plant Physiology Laboratory, Jagellonian Institute, Cracow, Poland

Ball
    Ball Film Laboratories, 1256 Howard St., San Francisco, California

Bausch & Lomb
    Bausch and Lomb Optical Co., Film Distribution Service, 635 St. Paul St., Rochester 2, New York

Bishop

James F. Bishop, dist. by Classroom Film Distributors, Inc., 5620 Hollywood Blvd., Los Angeles 28, California

Bray

Bray Studios, 729 7th Ave., New York 19, N.Y.

Brice

Arthur T. Brice, Phase Films, 656 Austin Ave., Sonoma, California

Carnegie

Carnegie Institution of Washington, 1530 P Street, N.W., Washington 5, D.C.

Carousel

Carousel Films, Inc., 1501 Broadway, New York 36, N.Y.

Colburn

Colburn Film Distributors, 668 North Western Ave., Lake Forest, Illinois

Contemporary

Contemporary Films, 267 W. 25th St., New York 1, N.Y.

Coronet

Coronet Films, Coronet Building, 65 East South Water St., Chicago 1, Illinois

Disney

Walt Disney Productions, 500 S. Buena Vista Ave., Burbank, California

Dowling

Pat Dowling Productions, 1056 S. Robertson Blvd., Los Angeles 35, California

EBF

Encyclopædia Britannica Films, Inc., 1150 Wilmette Ave., Wilmette, Illinois

Emory

Emory University, School of Dentistry, Atlanta, Georgia

FON

Films of the Nations, Inc., 62 W. 45th St., New York 36, N.Y.

Handel

Handel Film Corp., 6926 Melrose Ave., Hollywood 38, California

IFB

International Film Bureau, 332 S. Michigan Ave., Chicago 4, Illinois

Indiana

Audio-Visual Center, Indiana University, Bloomington, Indiana or NET Film Service, Indiana University, Bloomington, Indiana

Iowa

Iowa State University of Science and Technology, Ames, Iowa

KB

Knowledge Builders, 625 Madison Ave., New York 22, N.Y.

Metropolitan

Metropolitan Life Insurance Co., 1 Madison Ave., New York 10, N.Y.

MGM

MGM Studios, Culver City, California

Minnesota
University of Minnesota, Film Production Division, Minneapolis, Minnesota
MLA
Modern Learning Aids, 160 E. Grand Ave., Chicago 11, Illinois
National Foundation
National Foundation for Infantile Paralysis, 120 Broadway, New York 5, N.Y.
Nebraska
University of Nebraska, Bureau of Audio-Visual Instruction, Extension Division, Lincoln 8, Nebraska
Ott
John Ott Pictures, Inc., P. O. Box 158, Lake Bluff, Illinois
Pennsylvania
Audio-Visual Aids Library, Psychological Cinema Register, The Pennsylvania State University, University Park, Pennsylvania
Pictura
Pictura Films, 41 Union Square W., New York 3, N.Y.
Roberts Rugh
Dr. Roberts Rugh, c/o Columbia Medical Center, 620 W. 168th St., New York 32, N.Y.
Squibb
E. R. Squibb & Sons, 745 5th Ave., New York, N.Y.
Sterling
Sterling Educational Films, 6 E. 39th St., New York 16, N.Y.
TFC
Teaching Film Custodians, Inc., 25 W. 23rd St., New York 18, N.Y.
Thorne
Thorne Films, Inc., 1229 University Ave., Boulder, Colorado
UCLA
University of California, Los Angeles, California
USC
University of Southern California, Department of Cinema, Los Angeles 7, California
USPH
National Medical Audio-Visual Facility, U. S. Public Health Service, Communicable Disease Center, Atlanta 22, Georgia
UW
United World Films, Inc., 1445 Park Ave., New York 29, N.Y.
VEC
Visual Education Consultants, 2066 Helena St., Madison 4, Wisconsin
Wilner
Wilner Films and Slides, P. O. Box 231, Cathedral Station, New York 25, N.Y.
YAF
Young America Films, Inc., 18 E. 41st St., New York 17, N.Y.

DIRECTORY OF SUPPLY HOUSES

There is no official BSCS supplier. The following supply houses listed below alphabetically are those that responded to a BSCS invitation to provide materials for this curriculum. They have indicated that they can supply selected items of good quality and in adequate quantity. However, the BSCS cannot assume responsibility for the quality of service. Where more than one address is given, you should contact the office nearest you.

Baltimore Biological Laboratory, Inc., 1640 Gorsuch Ave., Baltimore 18, Md.
Bausch & Lomb, Inc., 635 St. Paul St., Rochester 2, N.Y.
Calbiochem, 3625 Medford St., Los Angeles 63, Calif.
    161 W. 231 St., New York 63, N.Y.
    4930 Cordell Ave., Bethesda 14, Md.
Cambosco Scientific Co., 37 Antwerp St., Brighton 35, Mass.
Carolina Biological Supply Co., Burlington, N.C.
Central Scientific Co., 1700 N. Irving Park Rd., Chicago 13, Ill.
    3232 Eleventh Ave., N., Birmingham 4, Ala.
    160 Washington St., Somerville, Mass.
    6446 Telegraph Rd., Los Angeles, Calif.
    1040 Martin Ave., Santa Clara, Calif.
    237 Sheffield St., Mountainside, N.J.
    6610 Stillwell, Houston 32, Tex.
    6901 E. 12th St., Tulsa 12, Okla.
Clay-Adams, Inc., 141 E. 25th St., New York 10, N.Y.
Clinton Misco Corp., P. O. Box 1005, Ann Arbor, Mich.
Dale Scientific Co., Box 1721, Ann Arbor, Mich.
Elgeet Optical Co., Inc., 303 Child St., Rochester 11, N.Y.
Faust Scientific Supply Ltd., 5407 Schlueter Rd., Madison 4, Wis.
General Biological Supply House, Inc. (Turtox), 8200 S. Hoyne Ave., Chicago 20, Ill.
Harvard Apparatus Co., Inc., Dover, Mass.
The Lemberger Co., P. O. Box 482, Oshkosh, Wis.
Macalaster Scientific Corp., Educational Div., 253 Norfolk St., Cambridge 39, Mass.
Northern Biological Supply, P. O. Box 222, New Richmond, Wis.
Science Education Products, Div. of B-D Laboratories, Inc., San Carlos, Calif.
Scientific Products, Division American Hospital Supply, 1210 Leon Place, Evanston, Ill.
    5056 Peachtree Rd., Chamblee, Ga.
    101 Third Ave., Waltham 54, Mass.
    3713 N. Davidson St., Charlotte 5, N.C.
    1586 Frebis Lane, Columbus 6, Ohio
    2505 Butler St., Dallas 35, Tex.

17150 Southfield Rd., Allen Park, Mich.
12th Ave. and Gentry St., N. Kansas City 16, Mo.
3815 Valhalia Drive, Burbank, Calif.
1951 Delaware Pkwy, Miami 35, Fla.
3846 Washington Ave., N. Minneapolis 12, Minn.
4408 Catherine Aye., Metairie, La.
40–05 168 St., Flushing 48, Long Island, N.Y.
150 Jefferson Dr., Menlo Park, Calif.
14850 N. E. 36 St., Bellevue, Wash.
3175 V. St., N. E., Washington 18, D.C.
Sherwin Scientific Co., N. 1112 Ruby St., Spokane 2, Wash.
Travel-Lab Science Co., P. O. Drawer M, Manor 1, Tex.
Van Waters & Rogers, Inc., P. O. Box 5287, Denver, Colo.
BKH Division:
P. O. Box 3200, San Francisco 19, Calif.
850 S. River Rd., W., Sacramento, Calif.
650 W. 8 St., S., Salt Lake City 4, Utah
313 Kamakee St., Honolulu 14, Hawaii
Scientific Supplies Co. Division:
600 S. Spokane St., Seattle 4, Wash.
3950 N. W. Yeon Ave., Portland 10, Ore.
Braun Division:
1363 S. Bonnie Beach Pl., Los Angeles 54, Calif.
P. O. Box 1391, San Diego 12, Calif.
P. O. Box 1431, Phoenix, Ariz.
2030 E. Broadway, Tucson, Ariz.
324 Industrial Ave., N. E., Albuquerque, N.M.
6980 Market Ave., El Paso, Tex.
Ward's Natural Science Establishment, Inc., P. O. Box 1712, Rochester 3, N.Y.
P. O. Box 1749, Monterey, Calif.
Welch Scientific Co., 1515 Sedgwick, Chicago 10, Ill.
331 E. 38th St., New York 16, N.Y.

FORMULAS FOR SOLUTIONS AND MEDIA

Aceto-carmine stain

45 ml glacial acetic acid
55 ml distilled water
carmine (powdered) to excess

Dissolve the carmine in the acetic acid. Add the water and bring to a boil in a well-ventilated room. Cool and filter. Add 2 drops of ferric chloride ($FeCl_3$) before using. Avoid excess ferric chloride.

Agar, glucose

2 g agar
0.5 g glucose-1-phosphate
100 ml distilled water

Boil until foam becomes quite coarse before pouring into Petri dishes to harden.

Agar, inorganic nutrient

1 liter inorganic nutrient solution
15 g agar

Heat until dissolved. Sterilize and pour into sterile Petri dishes or store in sterile stoppered test tubes (10–15 ml).

Agar, non-nutrient

15 g agar
1000 ml distilled water

Mix and heat until agar dissolves. Autoclave at 15 pounds pressure for 15 minutes.

Agar, nutrient

3 g beef extract (Difco B126)
5 g peptone (Difco 120)
15 g agar
1000 ml distilled water

Heat the distilled water until it is hot, but not boiling. Dissolve agar in the water, add peptone and beef extract. Sterilize in autoclave or pressure cooker for 15 minutes at 15 pounds pressure (121° C). Pour into sterile Petri dishes or test tubes while still hot.

Agar, starch

- 10 g powdered starch
- 10 g agar
- 980 ml distilled water

Mix and autoclave before use.

Agar, sugar nutrient

Add 10 g of a 1% solution of sucrose to nutrient agar solution (*see* above) when peptone is added.

Beef bouillon

Dissolve one beef bouillon cube in 500 ml of warmed distilled water. Filter before use to remove fat and sediment.

Benedict's solution

Benedict's solution can be purchased ready-made. It will detect 0.15% to 0.20% dextrose by forming a yellow-red precipitate of cuprous oxide when heated with the "unknown."

- 173.0 g sodium or potassium citrate
- 200 g crystalline sodium carbonate (Na_2CO_3), or 100 g anhydrous Na_2CO_3
- 17.3 g crystalline copper sulfate ($CuSO_4$)
- 1000 ml distilled water

Dissolve the carbonate and citrate in 700 ml of the water. Warm it to speed solution. Filter. Dissolve the $CuSO_4$ in 100 ml of water. Slowly add the 100 ml solution to the 700 ml solution while stirring constantly. Cool. Add water to make 1000 ml.

Benzalkonium chloride

Use as surface disinfectant in 1:1000 to 1:40,000 aqueous solutions. It is marketed as:

- Zephiran chloride
- Zephirol
- BTC
- Benirol
- Germitol
- Roccal
- Drapolene
- Cequartyl
- Germinol
- Rodalon
- Osvan

Bial's reagent

CAUTION: *Corrosive mixture!*

- 0.2 g of orcinol
- 100 ml concentrated sodium chloride solution

Biuret reagent

25 ml of 3% solution of copper sulfate ($CuSO_4$)
1000 ml of 10% solution of potassium hydroxide (KOH)

Bromthymol blue indicator

0.1 g bromthymol blue
16.0 ml *N*/100 sodium hydroxide (NaOH) pellets
234 ml distilled water

Grind bromthymol blue with sodium hydroxide in a mortar. CAUTION: *Handle sodium hydroxide with care—it is caustic!* Dilute to 250 ml with distilled water. This indicator changes from yellow to blue in the pH range of 6.0 to 7.6.

For 0.04% solution, use

0.2 g bromthymol blue
250 ml 95% alcohol
250 ml distilled water

Mix alcohol and water and put in graduated cylinder. Add bromthymol blue and stir until dissolved.

Broth, nutrient (beef broth)

Use the formula for nutrient agar, omitting the agar. Dissolve beef extract and peptone in water. Sterilize as for nutrient agar in the containers in which it will be used.

If you buy the ready-mixed nutrient, use the following proportions:

8 g Difco B3 nutrient broth medium
1000 ml distilled water

Dissolve the medium in the water. Sterilize as for nutrient agar in the containers in which it will be used.

Carmine solution

0.65 g carmine
10 ml water

The particles are insoluble in cold water and make a particulate solution for observing movement under the microscope.

Carnoy's solution

33 ml glacial acetic acid
33 ml absolute alcohol
33 ml chloroform
25 g mercuric chloride ($HgCl_2$)

CAUTION: *Highly toxic and inflammable. Be especially cautious with mercuric chloride. Avoid contact with the skin.* Combine ingredients just before use.

Carnoy's solution (modified)

60 ml absolute alcohol
30 ml chloroform
10 ml glacial acetic acid

Combine the ingredients just before use. CAUTION: *Inflammable!* (Mercuric chloride has been eliminated because of its highly toxic properties.)

Ceraphyl solution

15 g Ceraphyl
0.01 g magnesium sulfate ($MgSO_4$)
1 liter distilled water

Ceraphyl can be obtained in pint bottles from Ceraphyl Industries, Kansas City, Mo. The above formula gives a stock solution for protozoan culture. This medium, when inoculated with *Aerobacter aerogenes* is even more effective as a culture medium for protozoa.

Chloride solution, *see* Sodium chloride solution.

Congo red vital stain

0.5 g Congo red
100 ml distilled water

Stir Congo red into the water until all dye is dissolved. Filter to remove any large particles.

Crystal violet stain

2 g crystal violet (gentian violet)
20 ml 95% ethyl alcohol
180 ml distilled water

Dissolve the crystal violet in the alcohol and add the water. This is a simple substitute for the Gram stain. Results, however, are not consistent.

***Drosophila* medium**

See *Teacher's Manual*, Exercise 30-1 for mixing and use.

95 g corn meal
15 g agar
7.5 g dry brewers' yeast
75 ml molasses
75 ml corn syrup
850 ml water
5 ml 10% solution of *ortho*-phenylphenol (*o*-phenylphenol) in 95% alcohol

Drosophila medium (Glass)

See *Teacher's Manual*, Exercise 30-1 for mixing and use.

540 ml water
50 g corn meal
25 g rolled oats
50 g dextrose
5 g brewers' dry yeast
3 g agar
3 ml 0.5% propionic acid

Drosophila medium (Hollander)

See *Teacher's Manual*, Exercise 30-1 for mixing and use.

3 parts by volume sugar
2 parts by volume corn meal
1 part by volume brewers' yeast
1 part by volume granulated agar

FAA (formaldehyde, alcohol, acetic acid) solution

50 ml 95% ethyl alcohol
2 ml glacial acetic acid
10 ml 40% formaldehyde
40 ml distilled water

Fehling's solution

SOLUTION A: 34.65 g copper sulfate ($CuSO_4$)
500 ml distilled water

SOLUTION B: 125 g potassium hydroxide (KOH)
173 g potassium sodium tartrate
500 ml distilled water

Test for simple sugars by adding equal amounts of Solution A and Solution B above to "unknown" and heat. If sugars are present a yellow-red precipitate forms. Can be purchased ready-made.

Ferric chloride solution (1%)

1 g ferric chloride ($FeCl_3 \cdot 6H_2O$)
100 ml distilled water

Formalin solution (5%)

1 part commercial formaldehyde (37–40%)
19 parts water

CAUTION: Always wash hands thoroughly after using formaldehyde. Avoid inhalation of vapors.

Giemsa stain

0.5 g Giemsa powder
33 ml glycerin
33 ml absolute methyl alcohol (acetone-free)

It takes 1 to 2 hours to dissolve the powder in the glycerin. Then add the acetone-free absolute methyl alcohol. This stock solution is diluted 1:10 with distilled water before using.

Glucose solution (0.1%)

0.1 g glucose
100 ml distilled water

For 5% solution, use 5 g of glucose. For 80%, add sugar to 100 ml distilled water until solution is saturated. Fill graduated cylinder with 80 ml of saturated sugar solution and fill with distilled water to 100 ml mark.

Gram's staining technique

Ammonium oxalate-crystal violet solution

SOLUTION A:

2 g crystal violet (90% dye content)
20 ml ethyl alcohol (95%)

SOLUTION B:

0.8 g ammonium oxalate
0.80 ml distilled water

Mix Solutions A and B.

SOLUTION C: (MORDANT)

1 g iodine
2 g potassium iodide (KI)
300 ml distilled water

Procedure: Smear the culture to be stained on a slide and fix it by passing the slide several times through a flame. Add Solutions A and B to slide and let stand for 1 minute. Wash in tap water. Flood with Solution C and allow to stand for 1 minute. Wash again in tap water and blot dry. Decalorize 30 seconds by gentle movement in 95% alcohol and blot dry. Counterstain 10 to 30 seconds in safranin (*see* Safranin, p. 278). Wash in tap water, blot dry, and examine under the microscope.

Iodine solution, dilute (Lugol's solution)

10 g potassium iodide (KI)
100 ml distilled water
5 g iodine crystals

Dissolve potassium iodide in distilled water and add iodine. For delicate work, this solution may need to be diluted further.

Iodine–potassium–iodide solution (Gram's iodine)

3 g potassium iodide (KI) dissolved in 25 ml distilled water.
0.6 g iodine

Add the iodine to the KI solution and stir until dissolved. Add sufficient distilled water to make 200 ml solution. Store in dark bottle. Can be used wherever iodine stain is called for.

Intestinal juice (artificial)

1 g pancreatin
1 ml 1% solution calcium chloride ($CaCl_2$)
25 ml 0.2 *M* solution potassium monohydrogen phosphate (K_2HPO_4)
11.8 ml 0.2 *M* solution sodium hydroxide (NaOH)

Lugol's solution, *see* Iodine solution, dilute

Methyl cellulose

10 g methyl cellulose
90 ml water

Dissolve methyl cellulose in water. Add 1 drop to a drop of protozoa culture to provide a thick medium through which the organisms move slowly.

Methylene blue (stock solution)

Dissolve 1.48 g methylene blue dye in 100 ml of 95% ethyl alcohol.

Ninhydrin reagent

0.05 g ninhydrin
2 ml collidine (2,4,6-trimethylpyridine)
50 ml absolute ethyl alcohol
0.5 ml glacial acetic acid

This may be more easily purchased in ready-made spray can form from a supply house. CAUTION: *Because of its extremely toxic fumes, this reagent should always be used under a hood or beside an open window.*

Nitrogen-free nutrient

0.8 g potassium monohydrogen phosphate (K_2HPO_4)
0.2 g potassium dihydrogen phosphate (KH_2PO_4)
0.2 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$)
0.1 g calcium sulfate ($CaSO_4$)
0.01 g ferric sulfate ($Fe_2(SO_4)_3$)
1000 ml distilled water

Dissolve the salts in the water, mixing thoroughly.

Pentose sugar solution (0.25%)
0.25 g of xylose or other pentose sugar
100 ml distilled water

Phenol
1 g phenol crystals
15 ml water

Dissolve crystals for saturated solution. Keep container well closed and protected from light. CAUTION: *Do not handle crystals with bare hands. Death has resulted from as little as 1.5 g absorption.* Never apply to large portions of the body surface. Dilute before using. Use only as disinfectant and rinse surfaces with water after use.

Phenolphthalein solution
1 g phenolphthalein powder
40 ml ethyl alcohol
120 ml distilled water

Phloroglucinol (1,3,5-trihydroxybenzene)
Mix 1 g phloroglucinol in 10 ml of 95% alcohol.

Physiological saline, *see* Saline solution

Potassium iodide, *see* Iodine–potassium–iodide solution

PTC papers (phenylthiocarbamide)
500 mg phenylthiourea (PTC)
1000 ml distilled water
filter-paper strips

Dissolve PTC in water. At room temperature this takes about 24 hours. Soak sheets of filter paper in this solution and hang them up to dry. Cut into half-inch squares and store in covered containers or envelopes until ready to use. *N.B. At higher concentrations nontasters will also react!*

Ringer's solution (amphibian)
0.14 g potassium chloride (KCl)
6.50 g sodium chloride
0.12 g calcium chloride ($CaCl_2$)
0.1 g sodium hydrogen carbonate ($NaHCO_3$)
1000 ml distilled water

Dissolve salts in water, mixing thoroughly.

Safranin
10 ml safranin (2.5% solution in 95% alcohol)
100 ml distilled water

Saline solution (5%)

5 g sodium chloride
95 ml distilled water

For physiological saline solution (0.9%), use 0.9 g sodium chloride in 100 ml distilled water.

Silver nitrate solution (10%)

10 g silver nitrate
100 ml distilled water

Sodium chloride solution (0.1%)

0.1 g sodium chloride
100 ml distilled water

For 5% solution, put 5 g sodium chloride in 100 ml distilled water. For 95%, make a saturated solution of sodium chloride. Put 95 ml into a graduated cylinder and fill to 100 ml mark.

Sodium hydroxide (0.04%)

4 g sodium hydroxide (NaOH) pellets
1000 ml distilled water

Take 10 ml of above solution and add to 90 ml of distilled water for 100 ml 0.04% NaOH.

Starch solution

Mix 1 g dry cornstarch with 3 ml of distilled water. Then add 97 ml of boiling distilled water and stir into the starch paste. Cook for 2 minutes, stirring constantly. Cool and store.

Sugar solution, *see* specific sugar solutions, e.g. glucose, pentose, etc.

Urea (0.1 *M*)

0.6 g urea
100 ml distilled water

Xanthydrol

10 g of xanthydrol
113 ml absolute methyl alcohol

Shake vigorously; then let stand for 2 days. Filter into a narrow-mouthed, amber-colored bottle, using dry filter paper in a funnel. Stopper tightly.

Yeast suspension

1 g peptone
½ packet dry yeast
500 ml 5–10% molasses in distilled water

Culture for at least 12 hours at 25°–30° C.

UNITS OF MEASUREMENT

THE METRIC SYSTEM

LINEAR MEASURE

10 millimeters = 1 centimeter
10 centimeters = 1 decimeter
10 decimeters = 1 meter
10 meters = 1 decameter
10 decameters = 1 hectometer
10 hectometers = 1 kilometer

LIQUID MEASURE

10 milliliters = 1 centiliter
10 centiliters = 1 deciliter
10 deciliters = 1 liter
10 liters = 1 decaliter
10 decaliters = 1 hectoliter
10 hectoliters = 1 kiloliter
1000 milliliters = 1 liter

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SQUARE MEASURE

100 square millimeters = 1 square centimeter
100 square centimeters = 1 square decimeter
100 square decimeters = 1 square meter
100 square meters = 1 square decameter
100 square decameters = 1 square hectometer
100 square hectometers = 1 square kilometer

WEIGHTS

10 milligrams = 1 centigram
10 centigrams = 1 decigram
10 decigrams = 1 gram
10 grams = 1 decagram
10 decagrams = 1 hectogram
10 hectograms = 1 kilogram
10 kilograms = 1 quintal
10 quintals = 1 ton

COMMON CONVERSION FACTORS

ENGLISH TO METRIC UNITS

LINEAR

1 inch = 25.4001 millimeters
1 inch = 2.54001 centimeters

1 foot = 0.304 meter
1 yard = 0.914 meter
1 mile = 1.609 kilometers

SQUARE

1 square inch = 645.16 square millimeters
1 square inch = 6.4516 square centimeters
1 square foot = 0.092 square meter
1 square yard = 0.836 square meter
1 square mile = 2.59 square kilometers
1 acre = 0.404 hectare

CUBE

1 cubic inch = 16,387.2 cubic millimeters
1 cubic foot = 0.0283 cubic meter
1 cubic yard = 0.764 cubic meter

CAPACITY

1 U.S. liquid ounce = 29.573 milliliters
1 U.S. liquid quart = 0.946 liter
1 U.S. liquid gallon = 3.785 liters

1 U.S. dry quart = 1.1012 liters
1 U.S. peck = 8.809 liters
1 U.S. bushel = 0.352 hectoliter

WEIGHT

1 troy pound = 0.822 avoirdupois pounds
1 grain = 0.064 gram
1 avoirdupois ounce = 28.349 grams
1 troy ounce = 31.103 grams
1 avoirdupois pound = 0.453 kilogram
1 troy pound = 0.373 kilogram
1 long ton = 1.01 metric tons = 1016.05 kilograms (2240 avoirdupois pounds or 2722 troy pounds)
1 short ton = 0.907 metric ton = 907.18 kilograms (2000 avoirdupois pounds or 2430 troy pounds)

KITCHEN MEASURE

1 common tumbler = ½ pint
60 drops = 1 teaspoon = 4.9 milliliters
3 teaspoons = 1 tablespoon = 14.7 milliliters
16 tablespoons = 1 cup
2 cups = 1 pint = 0.473 liter

METRIC TO ENGLISH UNITS

LINEAR

1 millimeter = 0.0393 inch
1 centimeter = 0.3937 inch
1 meter = 3.280 feet
1 kilometer = 0.621 mile

CAPACITY

4.9 milliliters = 1 teaspoon
14.7 milliliters = 1 tablespoon
30 milliliters = 1 fluid ounce
1 liter = 1.056 U.S. liquid quarts

WEIGHT

1 gram = 0.035 avoirdupois ounce
1 kilogram = 2.204 avoirdupois pounds

S.C.E.R.T., West Bengal
Library
Calcutta
25/3, B. C. Road